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NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. Paul R. Gorham

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INDUCTION AND RELEASE OF DORMANCY IN *UTRICULARIA VULGARIS* L.

by

© ROBERT D. WINSTON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Induction and Release of Dormancy in *Utricularia vulgaris* L.

submitted by Robert D. Winston

in partial fulfilment of the requirements for the degree of Master of Science in Plant Physiology.

Supervisor

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ABSTRACT

The dormancy section of the life cycle of *Utricularia vulgaris* was studied with respect to environmental cues, changing growth regulator patterns and correlated developmental changes. These three aspects of dormancy were investigated in terms of both the field situation and in controlled environmental conditions. The primary data taken included developmental changes in field-grown material which occurred during the period just prior to turion formation up to the point of dormancy release and active summer growth. Main developmental changes noted in relation to prevailing environmental conditions were turion formation and sprouting and the turion's potential at a given time for sprouting and growth in a controlled environment. The involvement of environmental cues was investigated using controlled conditions to obtain turion induction and sprouting of preformed turions. Freeze-hardiness was also examined. Growth regulator involvement was investigated by determining endogenous levels in field-grown material at various stages and also by the action of synthetic growth regulators on these stages.

It was found that turion formation occurred during a period of decreasing daylengths and high temperature. Short day treatments were found to induce turion formation in actively growing plants. This response was enhanced by low temperature. Extraction of endogenous growth regulators prior to and during turion formation revealed that abscisic acid (ABA)-like and bound gibberellin (GA_n)-like levels were high while free GA_n-like and auxin-like levels were low. ABA exogenously applied to actively growing plants was found to rapidly induce turion formation even under non-inductive environmental conditions.

Immediately following turion formation there was a brief stage called pre-dormancy, which was characterized by the ability of the turion to sprout if detached from the parent plant. The pattern of endogenous growth regulator levels was similar to that found just prior to turion formation.

Pre-dormancy was followed by innate dormancy. This stage of dormancy occurred when daylength was decreasing but temperatures were still relatively high. The stage was characterized by an inability of the turion to sprout except at high temperatures or with an exogenous kinetin treatment. It was also found that this forced sprouting would invariably be followed by re-instatement of dormancy. Endogenous growth regulator levels were similar to those found during pre-dormancy but showed trends toward decreasing ABA-like levels and increasing GA_n-like and auxin-like levels. Artificial chilling of material collected during this stage resulted in the same response found for naturally chilled turions in terms of sprouting but not in terms of potential for continued vegetative growth. With respect to the re-instatement of dormancy the artificially chilled turions responded as did those which were forced to sprout by a heat or kinetin treatment. The lack of potential for continued growth was investigated only by a preliminary experiment which indicated that exogenous indoleacetic acid (IAA) and GA_3 could promote continued growth.

As environmental conditions deteriorated and night freeze-ups became regular, innate dormancy was lost and was replaced by an environmentally imposed dormancy. During this stage sprouting was easily obtained under ideal conditions and was followed by active vegetative growth. At this time there was a low level of ABA-like

inhibitor and high levels of GA_n-like and auxin-like compounds. Turions collected during this stage could be maintained in a dormant condition by incubation with ABA even at high temperatures.

With the disappearance of permanent ice cover, turions sprouted rapidly and underwent a period of rapid vegetative growth. At this time the endogenous pattern of growth regulators consisted of a low level of ABA-like inhibitor, a high level of a free GA_n-like compound and a high level of an auxin-like component. On the basis of this study, it is concluded that dormancy in the submerged aquatic annual, *Utricularia vulgaris* is controlled by changes in patterns of growth regulator levels that are similar to those hypothesized for dormancy control in many terrestrial woody perennials.

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INTRODUCTION AND LITERATURE REVIEW

Bud dormancy is a condition of arrested shoot growth and development. For the purposes of this discussion the concept of bud dormancy will be limited to rest or apparent cessation of shoot growth which occurs as an adaptation to unfavorable environmental conditions. The bud is composed of a primordial shoot or shoots with greatly shortened internodes covered and protected by modified leaves (cataphylls) or stipules. In some species such as *Pinus ponderosa* and *Larix decidua* the entire leaf complement and internode number for the upcoming growth season are present in the dormant bud (in Romberger 1963). The generalized concept of dormancy can be more accurately defined in terms of the three stages of dormancy. The stages are determined by the structure's response to favorable environmental conditions. The first stage, termed summer dormancy (Doorenbos 1953) or pre-dormancy (Vegis 1964), occurs as the dormant structure initially forms. This typically occurs during a period when environmental conditions are normally conducive to growth but at a time of shortening day lengths. Bud break can only be obtained by removal of developed leaves indicating that these organs are acting to initiate and maintain growth inhibition through some transmissible means (Wareing 1969b). The second stage of dormancy has been termed true dormancy (Vegis 1964), winter dormancy (Doorenbos 1953), rest (Chandler in Samish 1954) or innate dormancy (Wareing 1969a). In this stage, the bud is dormant due to internal factors and is least likely to sprout if placed in favorable environmental conditions. Following this period the bud becomes increasingly inclined to sprout and in the third and final stage, termed after-rest (Chandler in Samish 1954), imposed dormancy

(Doorenbos 1953) or post-dormancy (Wareing 1969a), the bud is maintained dormant by unfavorable environmental conditions without which sprouting would occur.

The primary source of information concerning dormancy has come from work with woody plants. Organs analogous to terminal resting buds of woody plants are bulbs, tubers, rhizomes and turions. Turions, an example of which is a subject of this study, are homologous to buds of woody plants. It should be noted that a major difference between the buds of woody plants and the analogous resting organs of herbaceous plants is the degree of environmental exposure received. Buds are products of phanerophytes, the other structures are produced by cryptophytes (Raunkiaer 1934). Turion is a term used for a specialized structure formed by aquatic angiosperms which has a degree of survivability over periods of environmental stress. Hibernaculae of aquatic angiosperms vary in complexity from the thickened frond of *Spirodela polyrhiza* (Jacobs 1947) and the condensed apices of species such as *Elodea canadensis*, *Ceratophyllum demersum*, and *Potamogeton spp.* to the more modified turions of *Myriophyllum verticillatum*, *Utricularia spp.* and *Hydrocharis morsus-ranae* (Sculthorpe 1967 and Arber 1920). Turions approach buds of woody plants in terms of complexity in that outer leaves are specialized and often scale-like. As a rule, turions and dormant apices of aquatics, as initially formed, are denser than water and over-winter on the bottom. These hibernaculae escape ice inclusion and exist in moderate temperature fluctuations relative to buds of woody plants. Turions which are buoyant (notably *Utricularia spp.*) often become embedded in ice, are subjected to much more extreme environmental conditions and are more accurately described as structures of hemi-

cryptophytes. H. Gluck (in Arber 1920) reported that turions of *Utricularia vulgaris* survived up to 12 days embedded in ice whereas those of *Myriophyllum* and *Hydrocharis morsus-ranae* although having a similar degree of morphological specialization, were much more sensitive and survived a maximum of 10 days in ice.

Turions typically serve as overwintering organs and are formed, as are buds of woody plants, in anticipation of deleterious environmental conditions (Sculthorpe 1967 and Arber 1920). Turions also have been reported (K. Goeble and G. Gluck in Arber 1920) to form in response to nutrient deficiency and desiccation in *Myriophyllum verticillatum*, *Sagittaria sagittifolia* and *Utricularia minor*. Recent work by Maier (1973a) has shown that *Utricularia vulgaris* produces turions in response to desiccation. Turion formation in response to desiccation is interesting in view of recent findings which show that plant water stress results in increased abscisic acid levels (Zeevart 1971 and Wright 1977). It has also been reported that ABA induces the formation of floating type leaves in submerged plants of *Potagmogeton nodosus* suggesting that the water stress resulting from emergence is substituted for by ABA (Anderson 1978).

While the most obvious function of turions is stress survival they are equally well-adapted for vegetative propagation. Typically, a plant produces a turion at the end of each branch and upon death of the main plant all are released for dispersal. In many aquatic angiosperms sexual reproduction is infrequent or non-existent (Sculthorpe 1967). Vegetative reproduction in aquatic angiosperms is often very efficient and serves to widely disperse the population. An example of this can be seen in the plantlet formation of *Echinodorus paniculatus* on which plantlets form in abundance in the axis of the sterile infloresence (DeWit 1964). *Myriophyllum exalbescens* Fern., studied at Douglas

Lake, Michigan, was seen to be primarily propagated by turions (Weber 1972).

The turion-forming species used in this study, *Utricularia vulgaris* L. var. *americana* Gray (Lentibulariaceae) (Fassett 1940, Moss 1959) is a rootless, freely-floating, submerged aquatic angiosperm. Distribution is circumpolar with gaps in Iceland and Greenland (Hulten 1971). *U. vulgaris* occupies littoral and sublittoral zones of lakes and ponds of diverse trophic levels (Ceska and Bell 1973). In Alberta it is characteristic of ponds, sloughs and ditches (Moss 1959).

Shoots of *U. vulgaris* are from 20-180 cm in length. "Leaves" are pinnatafid, 2-9 cm in length and composed of 20-150 filiform segments (Ceska and Bell 1973). The genus has attained a high degree of morphological plasticity such that distinctions between stem and leaf structures are not ridgidly defined (Schulthorpe 1967). *U. vulgaris* has bladders which occur at branching points in the leaf. The bladders have a carnivorous function (Lloyd 1942) which is characteristic of other members of the family. Flowers occur on an emergent scape and there are 3-15 per scape (Fassett 1940). Turions of *U. vulgaris* are globular, heavily setose, tough and range in size from 1-30 mm (Sculthorpe 1967 and Ceska and Bell 1973).

Dormancy was thought by many early physiologists to be initiated autonomously by plants at a particular season. Early work done with *Fagus sylvatica* by L. Jost (in Romberger 1963) and G. Klebs (in Vegis 1964) showed that this was incorrect. Much work has been done since to implicate various external phenomena as cues in dormancy initiation. Factors such as high temperature (Vegis 1964), low temperature and short daylength (Wareing 1956) have been suggested as critical. It has been shown that in a majority of woody plants, bud dormancy, leaf fall and cessation of cambial activity are induced by short daylengths (Wareing

1969a). A list of woody plants which have been tested for daylength sensitivity in terms of dormancy control is given by Vince-Prue (1975). Short days have also been implicated in initiation of apical growth cessation and shoot tip abortion in *Salix* (Junttila 1976). A red, far-red reversible system implicating phytochrome has been found for dormancy induction of *Weigela florida* (Vince-Prue 1975), *Larix europaea* (R. Vander Veen in Wareing 1969a) and for cold acclimation of *Cornus stolonifera* and *Weigela florida* but not *Pyracanthus coccinea* (Williams *et al.* 1972). Daylength perception in woody plants has been clearly shown to occur in leaves, particularly the youngest developed (Waxman in Wareing 1969b and Wareing 1954). While shortened photoperiods often induce morphological changes associated with dormancy, the physiological changes which allow for freezing tolerance are not necessarily correlated. Frost resistance in apple is produced by shortening daylengths (Howell and Wieser 1970) but in *Citrus* (Yelenosky and Guy 1977) and *Cornus stolonifera* (van Huystee *et al.* 1967) chilling temperatures are also required.

Dormancy induction by short days is not universal. Dormancy can be induced by long daylengths enhanced by high temperatures in a desert liverwort, *Lunularia crucinata* (Schwabe and Nachmony-Bascomb 1963). In this plant's normal habitat, long daylengths and increasing temperatures precede a period of low rainfall. The response of this plant is mediated by phytochrome (Wilson and Schwabe 1964). It has also been suggested that dormancy of woody plants is induced by nutrient deprivation (Kozlowski 1966 and Preistly 1962) and inadequate soil water (Perry 1971).

Dormancy in aquatic plants has been studied much less than has dormancy in woody plants. Turion formation is also considered to be a function of environmental conditions preceding winter (Sculthorpe 1967

and Arber 1920). Dormancy induction by short days occurs in *Hydrocharis morsus-ranae* (Terras 1900 and Vegis 1955). Turions of *Spirodela polyrhiza* are induced in populations from northern temperate regions by short daylengths (Perry and Byrne 1969). Although not aquatic, the herbaceous hemicryptophye *Pinguicula grandiflora* (Lentibulariaceae) which inhabits marshy, wet areas exhibits dormancy induction as a response to short day treatments (Heslop-Harrison 1962). Turion formation in *Myriophyllum verticillatum* (Weber 1976b) results from a combination of both low temperature and short photoperiods. Weber (1976b) also found that long day pre-treatments enhanced turion induction by short days. Turions of *Utricularia vulgaris* form turions in response to desiccation (Maier 1973b).

In summary, therefore, induction of dormancy in plants from northern temperate regions is a function of short day photoperiods. This light response is probably mediated by the phytochrome system. In some species cool temperatures are required in conjunction with short photoperiods either to induce dormancy or to induce a secondary stage of dormancy such as cold hardiness. Other, non-periodic environmental factors such as nutrient deficiency and desiccation may be involved in dormancy induction in some species.

While the involvement of the environment in dormancy induction is recognized, the mechanisms of perception and translation of environmental factors into morphological and physiological changes are far from understood. Early hypotheses centered around nutrient control (Klebs in Doorenbos 1953) oxygen deprivation (Vegis 1964) and high temperature effects on metabolism (Vegis 1964). With the discovery of auxin and elucidation of a hormonal control theory of plant growth and development it was inevitable that auxin would be implicated in dormancy control. Early work (Bennett and Skoog 1938) showed that in some cases dormancy

could be broken with auxin treatment. Later work failed to establish an association between auxin levels and dormancy stage. Hemberg (1949, 1958) demonstrated that bud dormancy and sprouting in *Fraxinus excelsior* was associated with changes in the level of an acid-ether-soluble inhibitor but not with changes in auxin levels. Ensuing work has supported the involvement of this inhibitor, termed inhibitor β (Bennett-Clark and Kefford 1953). This inhibitor chromatographs at R_f 0.5-0.7 in isopropanol:ammonia:water (PAW,10:1:1). The most active component of the inhibitory region was identified as a sesquiterpenoid carboxylic acid and was given the trivial name abscisic acid (ABA). Changes in the levels of inhibitor β have been associated with short-day dormancy induction in *Acer pseudoplatanus* (Phillips and Wareing 1962), *Betula pubescens* (Kawase 1961) and *Salix viminalis* (Bowen and Hoad 1968). In the latter case the compound primarily responsible for the inhibition was positively identified as ABA. In *Ribes nigrum* and *Fagus sylvatica* high levels of ABA determined by gas-liquid chromatography (GLC) correlated well with dormancy induction (Wright 1975). However, GLC analysis of *Acer pseudoplatanus*, *Betula pubescens* and *Acer rubrum* placed in short-day, dormancy-inductive conditions did not show an increase in the levels of ABA (Lenton and Saunders in Wareing 1969a). In a study of *Myriophyllum verticillatum*, Weber (1976b) found a high level of inhibitor β in extracts of turions.

Exogenous application of ABA to actively growing tissue also implies a role for ABA in dormancy regulation. *Betula pubescens*, *Acer pseudoplatanus* and *Ribes nigrum* formed typical resting buds in response to continuous application of ABA for 2-3 weeks (El Antably, Wareing and Hillman 1967). ABA has also been found to influence turion formation in aquatic angiosperms. In *Spirodela polyrhiza* (Perry and Byrne 1969

and Stewart 1969), ABA induced turions even under non-inductive photoperiods. In *Myriophyllum verticillatum*, ABA did not induce turions but acted to enhance turion formation under marginally inductive environmental conditions (Weber 1976b). ABA does not induce dormancy in potato tubers (Blumenthal-Goldschmidt and Rappaport 1965).

It is apparent that ABA plays a very important role in the regulation of dormancy. Dormancy-inducing short-day treatments have been found to cause an increase in the levels of endogenous ABA in some species. Exogenous ABA application to actively growing tissue in non-inductive photoperiods induces dormancy in many plants. But, along with these observations, are those of plant systems in which ABA is apparently not involved.

Gibberellins have been found in many instances to increase in levels in plants exposed to long daylengths and decrease with short-day treatments (Vince-Prue 1975). Gibberellin levels are also found to increase in dormant tissue of some species in response to periods of natural and artificial chilling (El Antably in Wareing 1969a). Hardiness inducing conditions for a cultivar of *Medicago sativa* caused a decrease in gibberellin levels (Waldman *et al.* 1975). These authors also found that ABA application could both induce hardiness and decrease levels of gibberellin. That this decrease in gibberellin levels is due to inactivation by glucoside formation is suggested by work done with barley aleurone layers in which applied ABA induced gibberellin glucoside production (Nadeau *et al.* 1972).

Exogenous application of gibberellins (usually gibberellic acid (GA_3) is used) has been found to counteract the dormancy-inducing effect of short day treatments in *Rhus typhina* (Nitsch 1957). This

study also showed that gibberellin application caused a reduction in the activity of an endogenous inhibitor which normally increased with short-day treatments. GA_3 applications frequently overcome bud dormancy in woody plants (see Vegis 1965) and delay growth cessation and shoot tip abortion in *Salix pentandra* (Junttila 1976). GA_3 is also effective in overcoming dormancy in *Laportea* bulbils (Tanno 1977), turions of *Hydrilla verticillatum* (Steward 1968), turions of *Potamogeton nodosus* (Frank 1966), dormant apices of *Ceratophyllum demersum* (Best and Soekarjo 1976) and turions of *Myriophyllum verticillatum* after a cold treatment has been received (Weber 1976a). Dormant buds of some species are not released from dormancy by a gibberellin treatment. Buds of pear showed no response to gibberellin application (Brown *et al.* 1960) while it prolonged dormancy of *Vitis vinifera* (Weaver 1959) and *Prunus avium* (Brian *et al.* 1959) buds. Turions of *Myriophyllum verticillatum* were not responsive to GA_3 when in their deepest stage of dormancy (Weber 1976a).

Gibberellins have been found to play an important role in bud dormancy regulation. Evidence from studies of endogenous levels as they change in relation to short-day photoperiods, low temperature and ABA treatment as well as studies using exogenous application of gibberellins give support to this statement. However, as was found with studies of ABA involvement, exceptions to the majority of observations indicate that gibberellins are not uniquely or universally involved in dormancy control.

Another class of growth promotor, the cytokinins, has been implicated in bud dormancy. Endogenous levels of cytokinins have been found to correlate with stages of dormancy. High levels are detected

after a chilling period and low levels prior to dormancy induction. In *Betula papyrifera* and *Populus balsamifera*, cytokinin levels increased as sprouting occurred and were at a minimum in dormant tissue (Domanski and Kozlowski 1968). In *Populus* x *robusta*, cytokinin levels measured in sap and bud tissue increased during both artificial and natural chilling periods to reach a maximum two weeks prior to bud burst (Hewett and Wareing 1973). These authors suggest a role for roots in cytokinin production. In xylem sap of *Salix viminalis*, cytokinin activity was high prior to spring growth and was at a minimum at the onset of dormancy (Alvim *et al.* 1976). Cytokinins have also been detected in the xylem sap of apple; high activity was correlated with spring bud burst and no activity was detected in dormant tissue (Luckwill and Whyte 1968). The possibility that roots act as the site of production is suggested by these studies of cytokinin activity in xylem sap. An effect of photoperiod has also been correlated with cytokinin activity in *Xanthium strumarium*, one short day caused a significant drop in activity measured in the leaf blade, bud and root exudate (Henson and Wareing 1974). In subsequent papers, evidence is presented by these authors for a shoot to root signal which modulates the rate of cytokinin production in the roots (Henson and Wareing 1977 a & b). There is evidence for one species, *Solanum andigena*, that cytokinin production can occur in bud tissue as well as roots (Wang and Wareing in Wareing *et al.* 1977).

The response of dormant tissue to exogenous cytokinin (usually kinetin or benzyladenine) application also provides evidence which supports a role in dormancy regulation. Synthetic cytokinins have been shown to cause sprouting in dormant buds of apple (Chvojka *et al.* 1962), *Vitis vinifera* (Weaver 1963) and *Pinus radiata* (Kumerow and Hoffmann

1963). As Vince-Prue (1975) notes this is particularly interesting as buds of apple, grape and conifers are notably insensitive to gibberellins. A recent report describes the induction of dormancy in *Pinus elliottii* by the direct application of 1.5 µg kinetin to the apex (Varnell and Vasil 1978). Another report contrary to typical promotive activity of cytokinins was one concerning the failure of kinetin to counteract apical growth cessation and shoot tip abortion in *Salix pentandra* (Junttila 1976). Synthetic cytokinins have been reported to break dormancy in buds of potato tubers (Tsukamoto 1972). Kummerow (in Romberger 1963) reported that kinetin was effective in breaking dormancy in turions of *Hydrocharis morsus-ranae*. In turions of *Spirodela polyrhiza* kinetin stimulated sprouting (Czopek 1964 and Stewart in Wareing and Saunders 1971). Buds of dormant bulbils of *Laportea* were stimulated to sprout by kinetin application (Tanno 1977). Benzyladenine blocks turion formation and breaks dormancy in non-chilled turions of *Myriophyllum verticillatum* (Weber 1976b).

In summary, there have been correlations made between endogenous cytokinin levels and states of dormancy which suggest that they play a role in dormancy control. Growth promoting photoperiods and chilling treatments have been associated with increases in endogenous cytokinin levels. Application of exogenous cytokinin to dormant organs in many cases causes a release from dormancy although numerous exceptions exist. As with ABA and gibberellins it appears that changes in cytokinin levels within plant tissue can be one of the factors in the growth substance set which control dormancy.

It is well accepted that these classes of plant growth substances play regulatory roles in dormancy control. When studying the significance of a single growth substance class in several species a general rule of inhibition or promotion emerges, always with exceptions. This inability

to assign function to a single class of substance indicates that control is exercised instead by dynamic interrelationships of more than one class. An example of this concept is shown by Khan (1975) in his model of seed dormancy in which growth substances classes are assigned preventive, permissive and primary roles of control. In such a model, dormancy can be induced in the absence of an inhibitor and dormancy release can occur in the presence of an inhibitor depending on the status of the other elements of the model.

The goals of the present study were to study the phenomenon of dormancy in *Utricularia vulgaris* L. Aspects of dormancy of primary interest were:

1) stage of dormancy
2) involvement of environmental factors in induction and release of dormancy
3) growth substance regulation of dormancy
4) physiological features associated with dormancy

U. vulgaris was chosen for the study because of the degree of specialization of the turion and because of the plant's rootless habit. The latter feature was desirable due to the possibility that cytokinins would be implicated in dormancy control, there being considerable evidence that cytokinins are produced in the roots. It was also thought that this rootless habit would eliminate culture complications arising from the necessity of providing a biphasic medium necessary for rooted species.

The approach used to determine depth of dormancy was to determine the potential for sprouting and subsequent growth under standardized conditions shown by tissue collected at various phenological stages.

Environmental involvement was investigated by obtaining information concerning phenological changes which occurred and relating it to

changing photoperiod and ambient temperature. The effect of artificial chilling was determined with turions collected throughout dormancy and chilled for various periods. Changes which occurred in these turions in terms of sprouting response, were compared to changes observed over the same period of time in naturally chilled turions. Growth chamber facilities were used to see if there was an effect on sprouting of turions in terms of photoperiod and temperature. Preliminary experiments were conducted concerning environmental induction of dormancy.

Growth substance involvement was approached in two ways; through extraction and assay of endogenous levels and by application of synthetic growth substances and determination of the response of tissue at various stages of the life cycle.

Physiological aspects of dormancy were studied by determining the changes in starch content during the study period and by measuring dark respiration in tissue from two stages of dormancy; innate dormancy and imposed dormancy.

Knowledge of dormancy in turion-forming plants is available for only three species; *Hydrocharis morsus-ranae*, *Spirodela polyrhiza* and *Myriophyllum verticillatum*. The object of this study was to provide information concerning the control of dormancy by environmental factors and growth regulators in turions of *Utricularia vulgaris* to enable comparisons with existing models and hypotheses to be made. It was felt that this multi-faceted approach would provide an accurate description of the various stages of dormancy in a species hitherto not well studied.

MATERIALS AND METHODS

Plant Collections

Utricularia vulgaris L. var. *americana* Gray (Lentibulariaceae) plants, turions and seeds were collected at intervals throughout the summer, fall and winter of 1977 from a shallow, eutrophic pond which was connected to a stream system. The pond was located in Parkland County, Alberta, Tp 51, R 26, approximately 10 km west of Edmonton in the NW corner of Highway 60 - Woodbend Road intersection. The pH of the water varied from neutral to slightly basic throughout the collection period. An analysis done on water collected in mid-summer is shown in Table 1. Plant material was in adequate supply in the pond; occurring predominantly in the shallower regions (< 0.25 m). Plants, turions and seeds were collected in this region prior to ice cover. After ice had formed, turions were collected at previously designated sites where caches had been located. Plants and turions were transported to the laboratory in an insulated container. Tissue to be extracted for growth substance analysis was thoroughly rinsed with distilled water, frozen in liquid nitrogen and stored at -25 C. Turions to be used for depth-of-dormancy studies were either tested immediately for sprouting potential or stored at 2-3 C in darkness. Seeds collected were taken from fruits, washed and stored wet or dry at 2-3 C in darkness.

Axenic Culture

It was apparent that confident interpretation of data on extracted growth substances would be facilitated by the use of axenic cultures of the plants in various stages of development. The first attempt to

Table 1. Water analysis of sample collected July 21, 1977 from the study site located at the Hwy. 60 - Woodbend Rd. intersection, Parkland County, Alberta.

Component		Amount (mg/ℓ)	Component		Amount (mg/ℓ)
Alkalinity ($CaCO_3$)	- Phenol.	4.4	Phosphate -	Total	0.25
	Total	228.4		Ortho	0.05
Total Residue		821.1		Meta + Poly	0.06
Iron		0.04		Organic	0.14
Chloride		14.2	Nitrogen -	Ammonia	0.16
Sulfate		200		Total Kjeldahl	3.27
Hardness ($CaCO_3$)	- Calcium	188		Organic	3.17
	Total	444		Nitrate	0.07
Silica		5.95		Nitrite	0.003

pH - 8.40

conductance - 880 μmhos

color (Pt units) - 197

obtain axenic cultures of *Utricularia* involved the use of turions. Surface sterilization by shaking turions in 1.23% or 0.66% solutions of NaOCl for 1 min resulted in either incomplete sterilization or dead plant material. Treatment of turions with various antibiotics (penicillin G, streptomycin sulfate, aureomycin) in distilled H_2O was tested but did not prevent contamination. These attempts showed that the mucilaginous turion with its tightly imbricated leaves would not provide a suitable source of plant inoculum for axenic culture.

One interesting observation did arise from this work. When plants were grown in a solution containing 333 units penicillin G·ml^{-1} a shortening of internodes was observed after one week's growth. Within 10 days this growth form was obviously involved in turion production. Plants cultured without penicillin did not form turions. This observation was confirmed by another culture with penicillin. No similar observation concerning the induction of dormancy or changing of growth patterns of higher plants by penicillin appears to have been reported before.

Seeds collected during June and July, 1977 were used in an attempt at axenic culture. In a sterile transfer room the seeds were rinsed with 95% ethanol, soaked in 2.5% NaOCl for 2 min. and rinsed once with sterile distilled water. Surface sterilized seeds were transferred to three different culture solutions: modified Hoagland's solution (Epstein 1972), modified White's medium (Dore Swamy and Mohan Ram 1969) and Medium II (Wetzel and Manny 1972) as shown in Table 2. Dilute Medium II (0.1 or 0.25 X) proved fatal to *Utricularia* and the modified White's medium promoted bacterial contamination. Dilute modified Hoagland's solution (0.1 or 0.25 X) proved the most effective and was used throughout the study. The plants were grown in controlled

Table 2. Three liquid media tested for suitability for the axenic culture of *Utricularia* seedlings.

Medium II - Wetzel & Manny's[1]		Modified Hoagland's Medium[2]		White's Medium Modified[3]	
Component	mg/ℓ	Compound	mg/ℓ	Compound	mg/ℓ
$CaCl_2$ (anhyd)	54	KNO_3	606.6	$Mg\ SO_4 \cdot 7H_2O$	360
NH_4Cl	3.82	$Ca(NO_3)_2 \cdot 4H_2O$	944.6	$Ca(NO_3) \cdot 4H_2O$	260
$MgSO_4 \cdot 7H_2O$	100	$NH_4H_2PO_4$	230.2	Na_2SO_4	200
Na_2CO_3	20	$MgSO_4 \cdot 7H_2O$	246.5	NaH_2PO_4	165
$Na_2S_iO_3 \cdot 5H_2O$	17	Fe EDTA (Fe)	5.0	KNO_3	80
KCl	30	H_3BO_3	11.44	KCl	65
$FeCl_3$ (anhyd)	1.38	$ZnSO_4 \cdot 7H_2O$	0.88	$MnSO_4 \cdot 4H_2O$	3.00
$ZnCl_2$	0.48	$CuSO_4 \cdot 5H_2O$	0.316	$ZnSO_4 \cdot 7H_2O$	0.5
H_3BO_3	0.40	$MnCl_2 \cdot 4H_2O$	7.24	H_3BO_3	0.5
Na_2Mo_4	0.254	Na_2MoO_3	0.072	Na_2MoO_3	0.025
$MnCl_2 \cdot 4H_2O$	0.0055			$CoCl_2$	0.025
$CoCl_2 \cdot 6H_2O$	0.0081			$CuSO_4 \cdot 5H_2O$	0.025
$CuCl_2$	0.0085			glycine	7.5
nitrilo triacetic acid	40			niacin	1.25
(ethylenedinitrilo) tetra-acetic acid disodium salt (EDTA)	0.08			thiamine · HCl	0.25
				pyridoxine · HCl	0.5
tris (hydroxymethyl) amino-methane	1.0			calcium pantothenate	0.25
				Fe-EDTA (Fe)	10

[1] Wetzel and Manny, 1972

[2] Epstein, 1972

[3] Doreswamy and Mohan Ram, 1969

environment facilities (Table 3 and Fig. 1) at 20 C with an 18-hour daylength. Seeds stored wet or dry gave at least 50% germination. However, those stored wet gave a higher percent germination and a faster response (6-8 days *vs* 4-6 weeks). The culture obtained in this way appeared, and remained, clean and seemingly contaminant-free but was found to be contaminated with bacteria when examined microscopically. These plants were too small to provide sufficient tissue for growth substance analysis but did provide a potential bioassay for growth substances extracted from field-collected *Utricularia*. An interesting observation was made about old cultures of these seedlings which had been kept without periodic transfer to fresh media. These consistently formed turions after a period of about 2 months. Transfer to fresh medium consistently induced sprouting.

Freeze Tolerance Tests

Low temperature tolerance was determined by subjecting sets of turions to decreasing temperatures and removing one set at certain temperature intervals. A set consisted of five turions placed in a polyethylene tube with 5 mls of distilled water. The tube was submerged in a methanol:water bath (Polytemp Model 90C, Polyscience, Niles, Illinois). In the tube with the set of turions to be removed last was a Cu-constantan thermocouple. Temperature was lowered at 0.23-0.26 C$\cdot$min^{-1} and sets were removed at approximately 5 C intervals for viability tests. Viability was determined by placing the turions in the LD chamber (Table 3, Fig. 1) and watching for signs of sprouting. Another indication of viability was whether the tissue remained floating or not. If tissue had been killed, cell integrity of the normally floating turion was destroyed

Table 3. Specifications of growth chambers used in this study.

Chamber Designation and abbreviation used	Make & Model	Lamps	PAR[1] μein·m^{-2}·s^{-1}	Day/Night, hrs.	Temperature, °C
Long Day (LD)	EGC[2] M-7	24 - Sylvania F96-T12-CW-VHO-fluor. 10 - 150 w - incand.	313	18/6	20, constant
Short Day (SD)	EGC M-3	20 - Sylvania F48-T12-CW-VHO-fluor. 6 - 150 w - incand.	157	12/12	20, constant
High Temp. (HT)	EGC M-3	20 - Sylvania F48-T12-CW-VHO-fluor. 6 - 150 w - incand.	330	16/8	30, constant
Soybean Callus (SC)	EGC M-3	2 - Sylvania F48-T12-CW-VHO-fluor.	12	24/0	26, constant
Other Bioassays (BC)	CE[3] 118L	8 - JetSet F15-T8-D-fluor.	149	24/0	25, constant

[1] PAR - photosynthetically active radiation, from 400-735 nm, received at plant level in chamber

[2] EGC - Environmental Growth Chambers, Chagrin Falls, Ohio

[3] CE - Controlled Environment, Ltd., Winnipeg, Manitoba

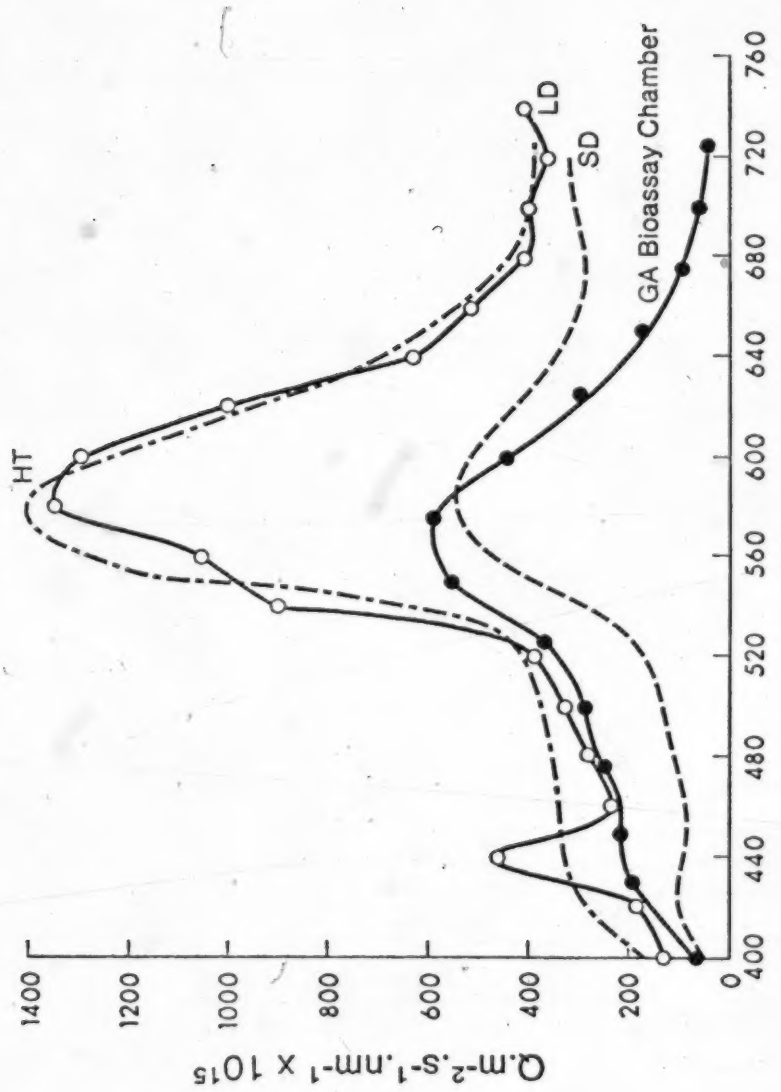

Fig. 1. Spectral energy distributions (SED) of four growth chambers used in this study.

and caused sinking within 12 hours.

Controlled Environment Studies

Studies using these facilities were done in order to determine the influence of photoperiod and temperature in dormancy induction and release. The facilities were also used for the maintenance of *Utricularia* cultures used in exogenous growth substance experiments and for the incubation of the various bioassays used. The specifications for each chamber are given in Table 3 and Figs. 1 and 2. New lamps were installed at the beginning of the experimental work and were not replaced unless malfunctioning. The period of use of these light sources was approximately 8 months. The spectral energy distribution (SED) and photosynthetically active radiation (PAR) were measured at approximately the mid-point of the study period. Both PAR and SED were determined with a Quanta Spectrometer QSM-2500 (Tectum Instruments, Umea, Sweden).

Determinations of Turion Depth of Dormancy

As soon as turion formation was observed in the field, periodic collections were begun. From a collection, ten turions were placed in a 125-ml erlenmeyer flask containing distilled water. Due to supply restrictions only one flask was placed in each chamber. Chambers used were SD, LD and HT (Table 3, Fig. 1 and 2). Approximately every three days each flask was scored for sprouted turions. Sprouting was defined as leaf reflexing and stem elongation. Sprouting and subsequent development was allowed to proceed for at least 24 days or until the flask was full of plant material. Sprouted plants were either transplanted for other studies or discarded.

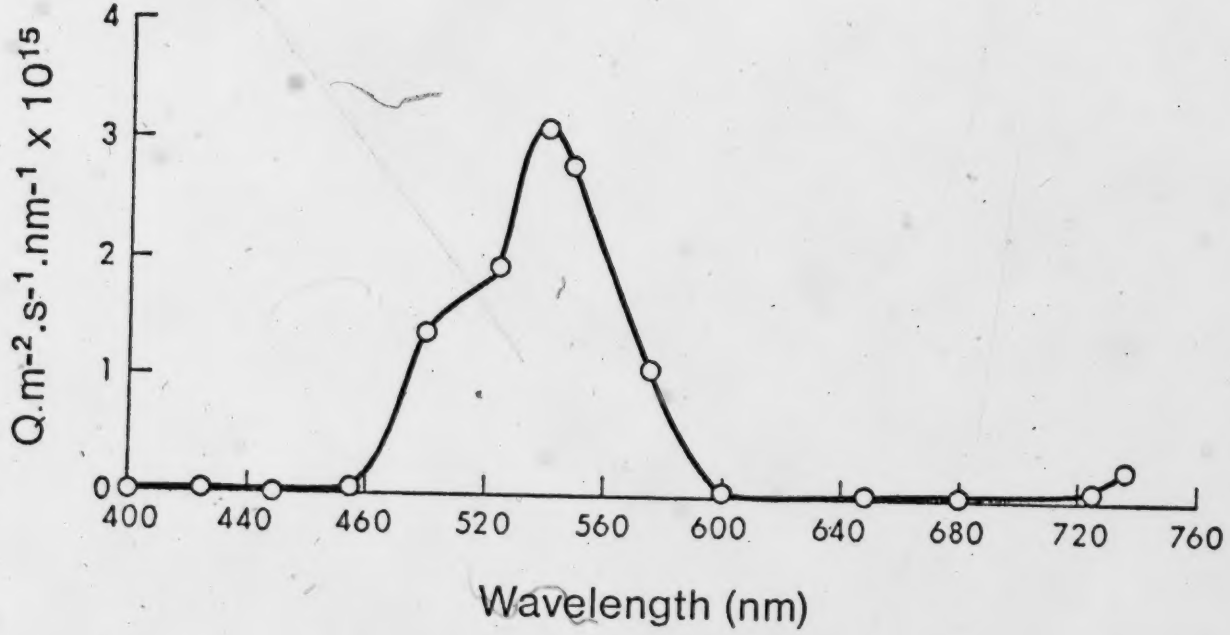

Fig. 2. Spectral energy distribution (SED) of the growth chamber used to culture soybean callus tissue used for the cytokinin bioassay.

Effects of Artificial Chilling on Dormancy Release

Turions not used in the depth of dormancy experiments were refrigerated (2-3 C) in the dark. The tissue was kept in this state until subsequent field collections were made, at which time 30 turions were taken from each artificially chilled batch and tested for depth of dormancy as described above.

Effect of Light on Turion Sprouting

From a batch of refrigerated, post-dormant turions 30 were taken. They were divided into three groups for treatment with red light, growth chamber light and no light. The red light was provided by one 75-watt incandescent reflector flood lamp filtered with a CBS 650 filter (Carolina Biological Supply, Burlington, North Carolina). The SED and PAR of this light treatment are shown in Fig. 3. The turions exposed to red light were irradiated for 15 min. All sets of turions were then placed in the LD chamber and scored for sprouting.

Dormancy Induction by Temperature and Photoperiod

Two experiments, both of a preliminary nature, were performed. The first experiment involved four flasks, each containing three apical shoot sections. Two flasks were placed in the LD chamber, one in the SD chamber and one in a chamber with short daylengths but with a temperature of 15 C and the light conditions as described for the HT chamber (Table 3, Fig. 1). The experiment ran for 36 days. The second experiment was similar to the first but had only one flask in the LD chamber and was terminated after 22 days.

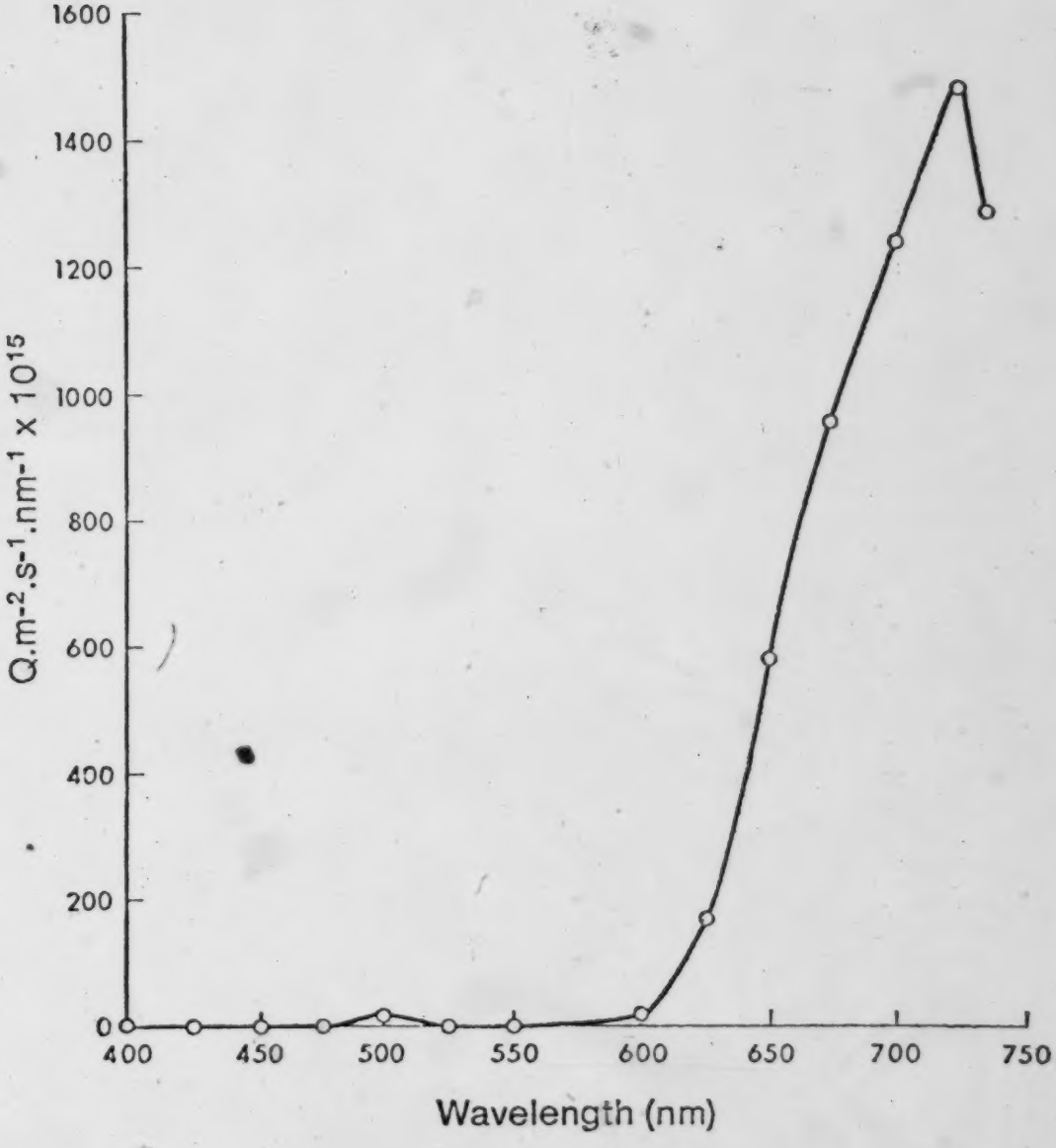

Fig. 3. Spectral energy distribution (SED) of the red light source used in the study of the effects of light on turion sprouting. PAR = 182 µein · m^{-2} · s^{-1}.

Growth Regulator Extraction

The Modified Shindy and Smith Method

The extraction procedure used was based on the method of Shindy and Smith (1975). The procedure followed the flow chart as shown in Fig. 4. Usually 50 g fresh weight of tissue was used for extraction. Re-distilled solvents were used throughout. The polyvinyl pyrrolidone (PVP) used in steps 3A and 4B was prepared by extensively washing the commerical product (Polyclar AT, GAF Corp., New York, N.Y.). The fines were decanted and a slurry remained. The slurry was used in step 3A and a 1.25 X 25 cm column was prepared for step 4B. Paper chromatography for all fractions was done in a descending fashion and was developed with 2-propanol:ammonia:water (PAW), (10:1:1). The paper chromatograms were dried overnight in a fume hood and assayed for the compounds of interest or stored at -15 C. Growth regulator assay was done by dividing the chromatogram into 10 R_f zones of equal size, keeping the origin separate. This provided 11 R_f segments to be analyzed. Samples to be analyzed for abscisic acid (ABA) via GLC were eluted from the paper at R_f 0.5-0.7 with diethyl ether. This was reduced to dryness, dissolved in methanol:ether (1:1) and spotted on a thin layer chromatography plate. The plate was 20 X 20 cm prepared with 0.25 mm U.V. indicating silica gel (Camag, Berlin, West Germany). The loaded TLC plates were developed in benzene:ethyl acetate:acetic acid (50:5:2). The zone co-chromatographing with marker ABA (± cis, trans ABA, Grade IV, Sigma Chemical Co., St. Louis, Missouri) was scraped off and eluted with ether. The ether was removed and the residue dissolved in 0.2 ml N, O-bis-(trimethylsilyl)-acetamide (BSA), (Grade I, Sigma Chemical Co., St. Louis, Missouri). The silylation was allowed to proceed for 30 min at room temperature and

A 1 - Freeze with liquid N_2; store at -25 C (50 g f.wt. tissue) : Infiltrate with 80% methanol containing 5 g/l NaH_2CO_3 at -5 C : Homogenize in Waring blender : Stir overnight at -5 C : Centrifuge at 6000 X g for 15 min : Extract pellet with solvent (1:1/2 w/v) at -5 C for 4 hrs : Centrifuge at 6000 X g for 15 min : Extract pellet with solvent (1:1/4 w/v) at -5 C for 4 hrs : Filter with Whatman No. 1 paper : Combine filtrate and supernatants : Reduce to aqueous at 35 C : Freeze and thaw : Centrifuge at 16,000 X g for 90 min.

2 - Adjust pH to 8.6 with 0.1 N HCl : Extract 3X with equal volumes ethyl acetate : Combine ethyl acetate fractions and dry over anhyd. Na_2SO_4 : Reduce to dryness at 35 C.

→ B (Fig. 4 cont.)

3 - Dissolve residue in H_2O and slurry with equal volume PVP slurry : Wash PVP 3X with equal volumes of H_2O.

4 - Adjust pH to 8.6 : Extract 3X with equal volumes H_2O saturated in butanol : Discard aqueous and reduce butanol to dryness at 50 C under vacuum.

5 - Dissolve residue in 50% methanol : Streak on 57 X 23 cm Whatman 3MM paper : Develop in 2-propanol : ammonia: H_2O (PAW 10:1:1).

6 - Bioassay basic ethyl acetate (BEA) fraction for cytokinins.

Fig. 4. Protocol in three parts, A, B, and C, of modified Shindy and Smith extraction procedure (SS). Redistilled solvents were used throughout and all work was conducted in subdued light.

B 3 - Adjust pH of aqueous fraction with 1N HCl to 2.8 : Extract 3X with equal volumes ethyl acetate.

→ C

4 - Reduce ethyl acetate to dryness : Dissolve residue in NH_4OH under N_2 : Pass through 1.25 x 25 cm PVP column : Wash column with 3X column volume of H_2O : Adjust pH of eluate to 2.8 : Extract 3X with equal volumes of ethyl acetate : Discard aqueous : Reduce ethyl acetate to dryness.

5 - Streak on 57 x 23 cm Whatman 3MM paper : Develop in 2 propanol : ammonia : H_2O (10:1:1) : Dry in fumehood overnight.

6 - Bioassay acidic ethyl acetate (AEA) fraction for free acids (ABA, GAn, IAA).

C 4 - Adjust pH of aqueous fraction to 7 with 1N NaOH : Store at 2-3 C overnight.

5 - Adjust pH to 11with NaOH : Heat to 65 C for 1 hr.

6 - Adjust pH to 2.8 with 2 N HCl : Extract 3X with equal volumes ethyl acetate : Discard aqueous : Reduce ethyl acetate fraction to dryness.

7 - Streak on Whatman 3MM paper 57 x 23 cm : Develop in 2 propanol : ammonia : H_2O (10:1:1) : Dry overnight in fumehood.

8 - Bioassay bound acidic ethyl acetate (B-AEA) fraction for bound acids (GA_n-, IAA-, ABA- conjugates).

Fig. 4 (Cont.). Protocol in three parts, A, B, and C. of modified Shindy and Smith extraction procedure (SS). Redistilled solvents were used throughout and all work was conducted in subdued light.

the products were either analyzed by GLC immediately or stored at -15 C.

Comparison of an Alternate Method with the Shindy and Smith Method

An alternate method for extracting growth regulators based on an ABA extraction procedure (Lenton, Perry and Saunders 1971) and a general growth regulator extraction procedure (Weber 1976b) was tested early in this study. Although there was good recovery of spiked ABA determined by GLC, poor recoveries were obtained as measured by bioassay. Also this alternate method had other problems related to gibberellin and cytokinin extraction and was therefore abandoned. A full description of this method and a comparison with the modified Shindy and Smith Method is given in the Appendix.

Growth Regulator Determinations

Gas-Liquid Chromatography

Instrumentation involved a Beckman GC-5 chromatograph and a Beckman 10" recorder equipped with a disc integrator (Beckman Instruments, Fullerton, California). The GC was equipped with flame ionization detectors and dual 1.5 m X 3 mm stainless steel coiled columns. Gas flow rates were determined with a soap bubble flowmeter. The nitrogen and carrier gas flow rates were 24 ml $\cdot$ min^{-1}, and the air flow rate was 300 ml $\cdot$ min^{-1}. Gases used were Linde Commercial Grade (Union Carbide Canada, Ltd., Toronto, Ontario). Both nitrogen and hydrogen gas lines were filtered with Nupro F-series 7 µ in-line filters (Nupro Co., Cleveland, Ohio). The columns were packed with 5% QF-1 coated 60/80 mesh acid-washed, DMCS treated Chromosorb W (Chromatography Specialists, Ltd., Brockville, Ontario). Chromatography consisted of injecting 3 µl of the BSA-silylated

sample into a column set at 100 C. The inlet temperature was set at 180 C, the line temperature at 180 C and the detector at 200 C. The linear temperature program was set to maintain isothermal temperatures for 4 min after which the column temperature was increased 25 C·min^{-1} for 4 min, reaching a maximum temperature of 200 C.

Bioassay

Bioassay methods which were considered and tested were the oat leaf chlorophyll retention bioassay for cytokinins (Weber 1973), the soybean callus bioassay for cytokinins (Miller 1967), the lettuce seed germination bioassay for inhibitors (Weber 1973), the oat coleoptile elongation bioassay for inhibitors and auxins (Weise and DeVay 1970), the lettuce hypocotyl elongation bioassay for gibberellins (Brian *et al.* 1964), the dwarf pea epicotyl elongation bioassay for gibberellins (Kohler and Lang 1963) and the α-amylase bioassay for gibberellins (Jones and Varner 1967).

The bioassays chosen for routine use were the oat coleoptile straight growth bioassay for inhibitors and auxins, the lettuce hypocotyl elongation bioassay for gibberellins and the soybean callus bioassay for cytokinins. Selection was based on how well described and widely used the bioassay was, the amount of variation obtained, bioassay specificity and its ability to detect growth regulators directly from the chromatography paper; thereby avoiding elution steps. With every bioassay, controls and three concentrations of a model synthetic growth regulator were simultaneously bioassayed to produce a standard curve. Synthetic growth regulators used were indole-3-acetic acid (Baker Grade, J. T. Baker Chemical Co., Phillipsburg, New Jersey), gibberellic acid (GA_3, 90%, Baker Grade, J. T. Baker Co.), kinetin (6-furfuryl amino-purine,

Nutritional Biochemicals Corp., Cleveland, Ohio), and abscisic acid (± cis, trans ABA, grade IV, approximately 95%, Sigma Chemical Co., St. Louis, Missouri). Confidence limits at the 0.05 level were calculated for each bioassay. Bioassays significantly beyond the control values were used to calculate synthetic growth regulator equivalents by use of the standard curve. Growth regulator equivalents were then calculated on a per gram dry weight basis to facilitate comparisons.

The Oat Coleoptile Straight Growth Bioassay for Auxins and Inhibitors

This bioassay involved soaking oat seeds (*Avena sativa* L. cv. Victory, United Grain Growers, Edmonton, Alberta) in aerated distilled water for 1 hour. Seeds were planted in vermiculite and grown in the dark at 25 C for 72 hours. With illumination provided by a green safe light (SED is given in Fig. 5) the coleoptiles were cut and 10 mm sections were taken 3 mm below the tip. The sections were floated on distilled water for a period of 0.5-1 hours. Ten sections were placed in 60 mm petri dishes containing test chromatogram sections (typically 3.8 X 4 cm), blank sections of chromatography paper for controls or sections of chromatography paper containing known amounts of synthetic substances as standards. Standards were applied as a methanol solution and the alcohol allowed to evaporate. To the petri dishes was added an incubation solution consisting of 2.73 g KH_2PO_4 and 10 g sucrose per liter. The incubation solution used for inhibitor bioassay also contained 1×10^{-5} M IAA. The incubation solution was added at least one hour prior to introduction of the coleoptile sections. The coleoptiles were incubated for periods of 18-24 hours with shaking. After incubation

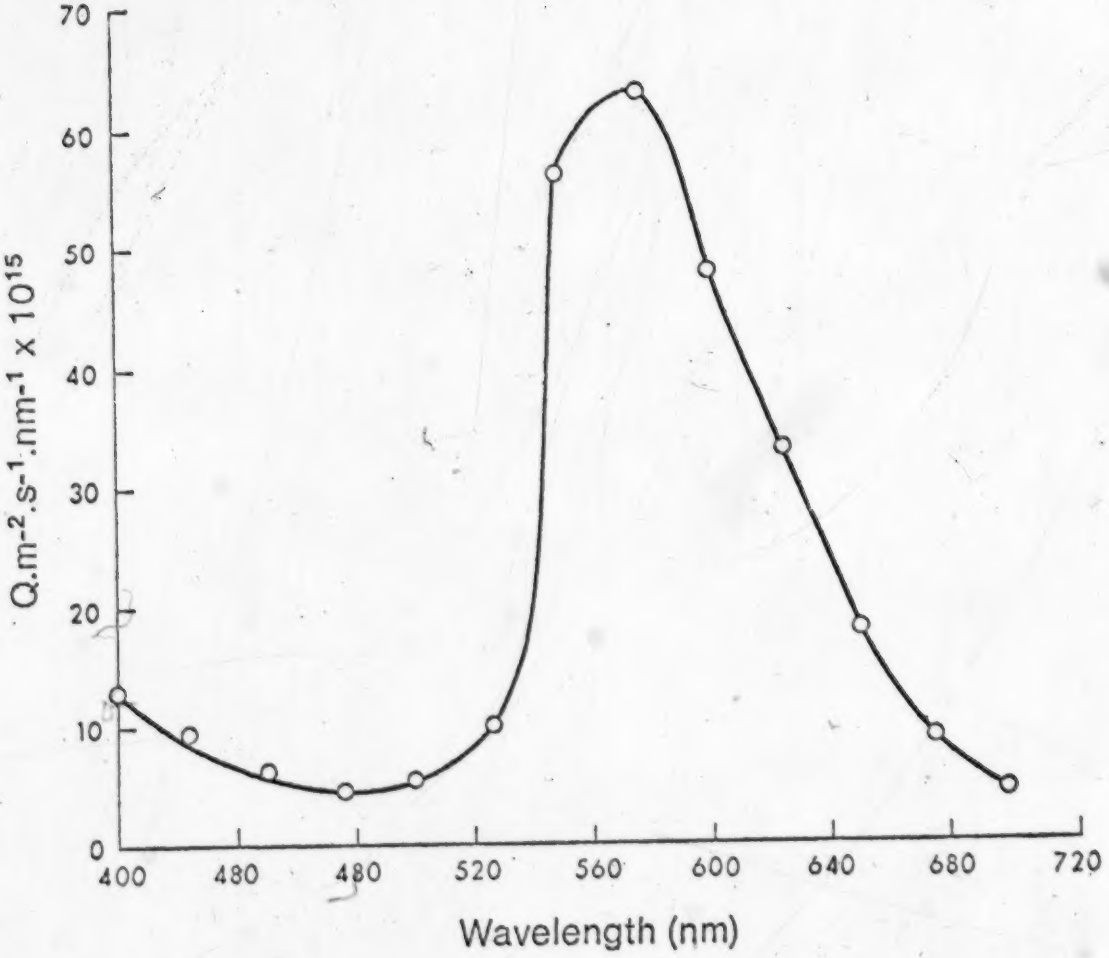

Fig. 5. Spectral energy distribution of green light source used as safe-light with oat coleoptiles for the auxin-inhibitor bio-assay.

they were measured to the nearest 0.5 mm using a dissecting microscope. An example of such a bioassay with two accompanying IAA standards and one ABA standard is shown in Fig. 6. One problem with this bioassay was that it was inhibited by an artifact of the chromatography system. This can be seen in both the inhibitor and auxin bioassay of developed, blank chromatography papersas shown in Fig. 7a, b. Fortunately, this inhibition predictably appeared at the solvent front and did not co-chromatograph with any compound of interest as far as is known. Therefore, inhibition at R_f 0.9-1 is not considered in interpretation of bioassay results.

Lettuce Hypocotyl Elongation Bioassay for Gibberellins

Lettuce seeds (*Lactuca sativa* L. cv. Grand Rapids, Robertson Seeds, Edmonton, Alberta) were germinated in the dark at 25 C for 36 hours. Seedlings were selected for radical uniformity and 10 were placed in 60 mm petri dishes. The petri dishes contained sections of chromatography paper with either test compounds, synthetic GA_3 or nothing. The incubation solution was 3 mls of 0.33 X Hoagland's solution. Chromatograph sections were soaked in the nutrient solution for at least 1 hour prior to lettuce seedling incubation. The bioassay was incubated in the gibberellin bioassay chamber (Table 3, Fig. 1) for 3 days. Measurements were taken immediately or the entire bioassay was refrigerated at 2-3 C and measurements were taken within 3 days (Reeve and Crozier 1975). The seedlings were placed between two microscope slides and hypocotyls measured to the nearest 0.5 mm. The bioassay is insensitive to IAA (Bailess and Hill 1971, Fig. 8) and only slightly sensitive to ABA (Fig. 8). A typical bioassay with two standards is shown in Fig. 9.

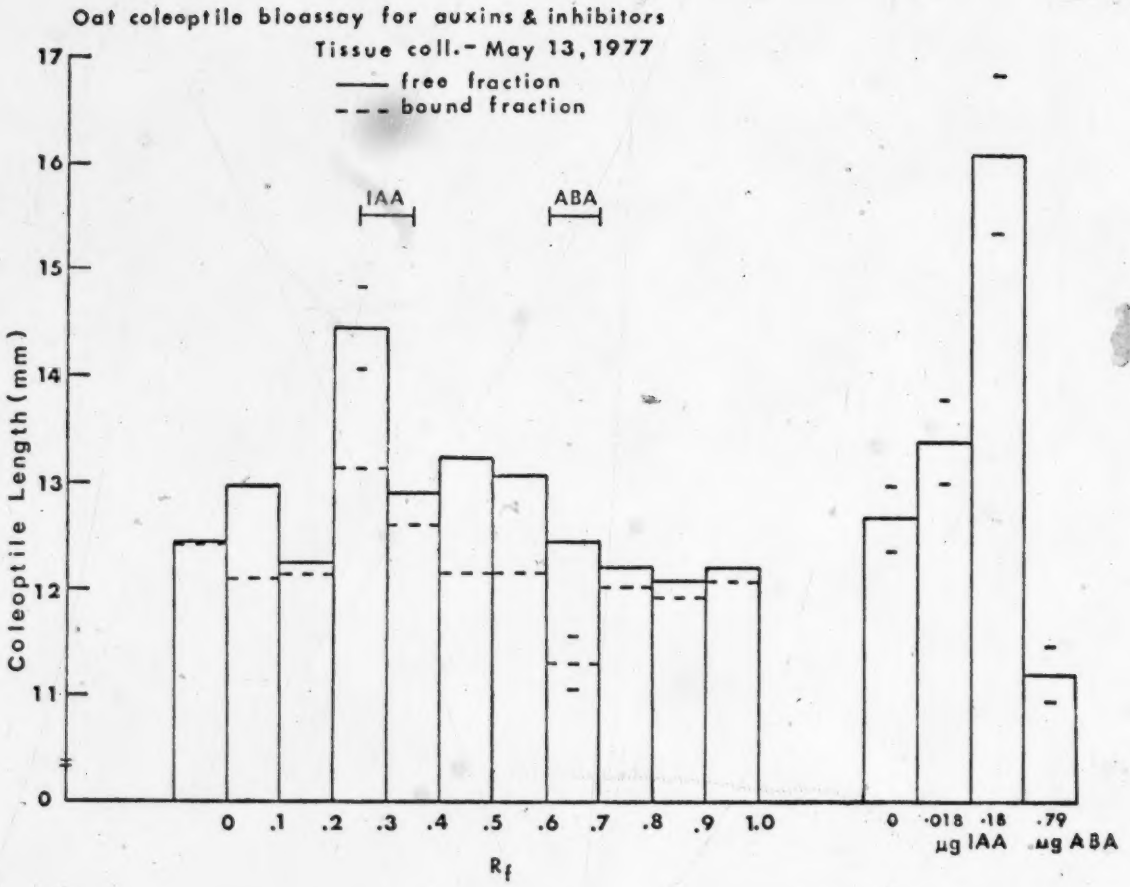

Fig. 6. Example of auxin-inhibitor bioassay of tissue extract with IAA and ABA standards. Confidence limits (p = 0.05) are shown as short lines above and below the means.

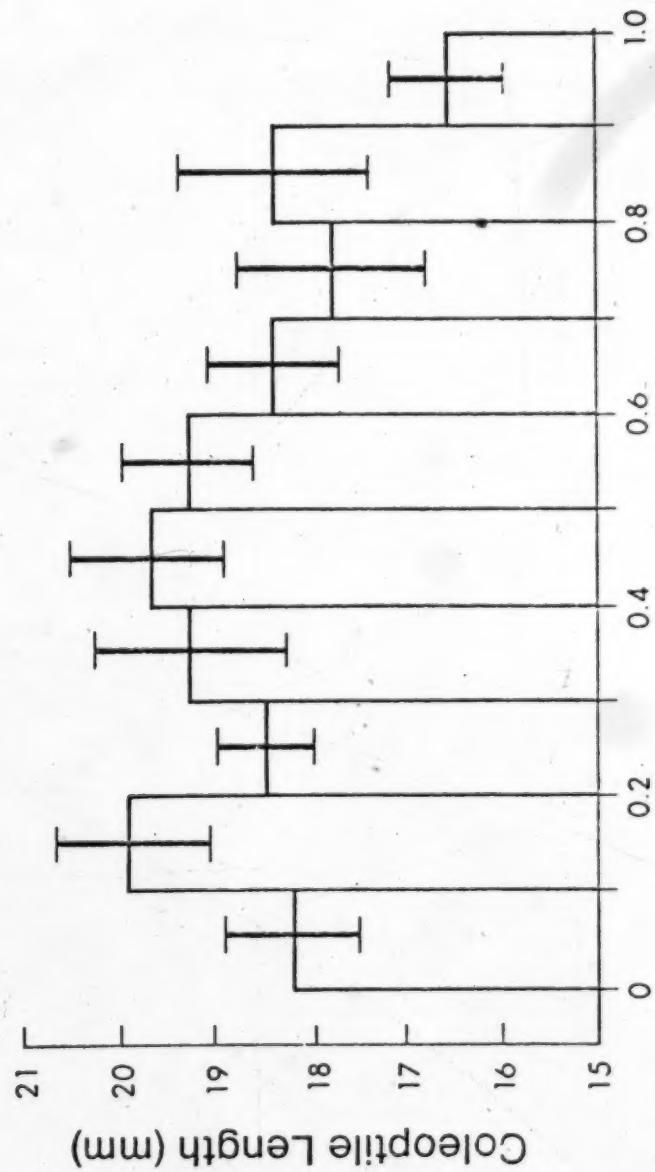

Fig. 7a. Results of an inhibitor oat coleoptile bioassay of Whatman 3MM paper developed in PAW. Confidence limits are for $p = 0.05$.

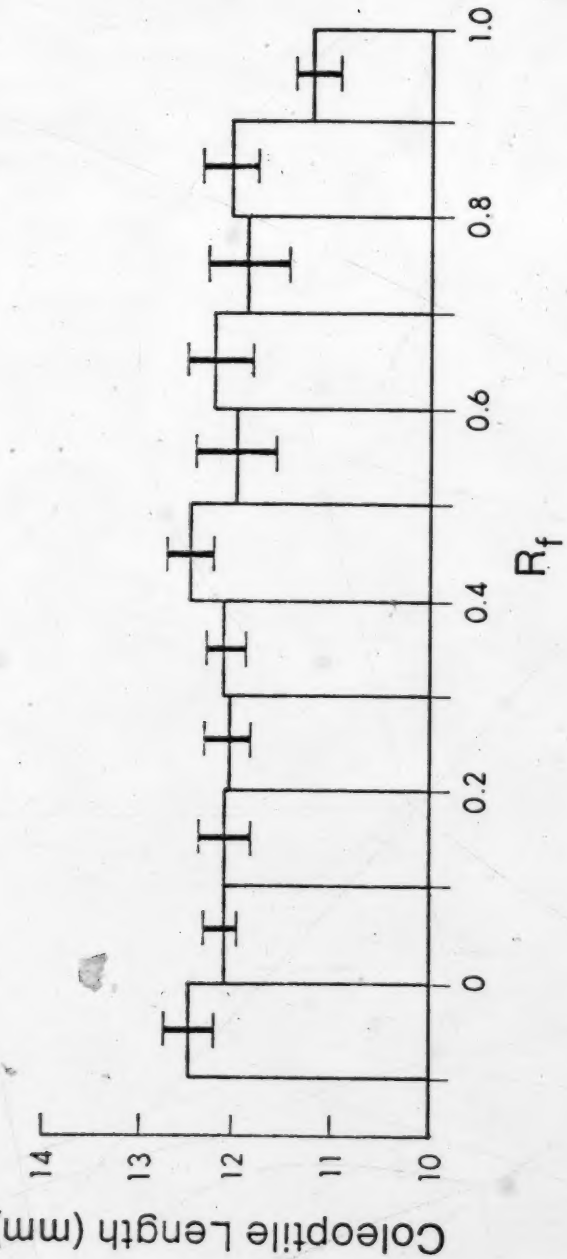

Fig. 7b. Results of an auxin oat coleoptile bioassay of Whatman 3MM paper developed in PAW. Confidence limits are for p = 0.05.

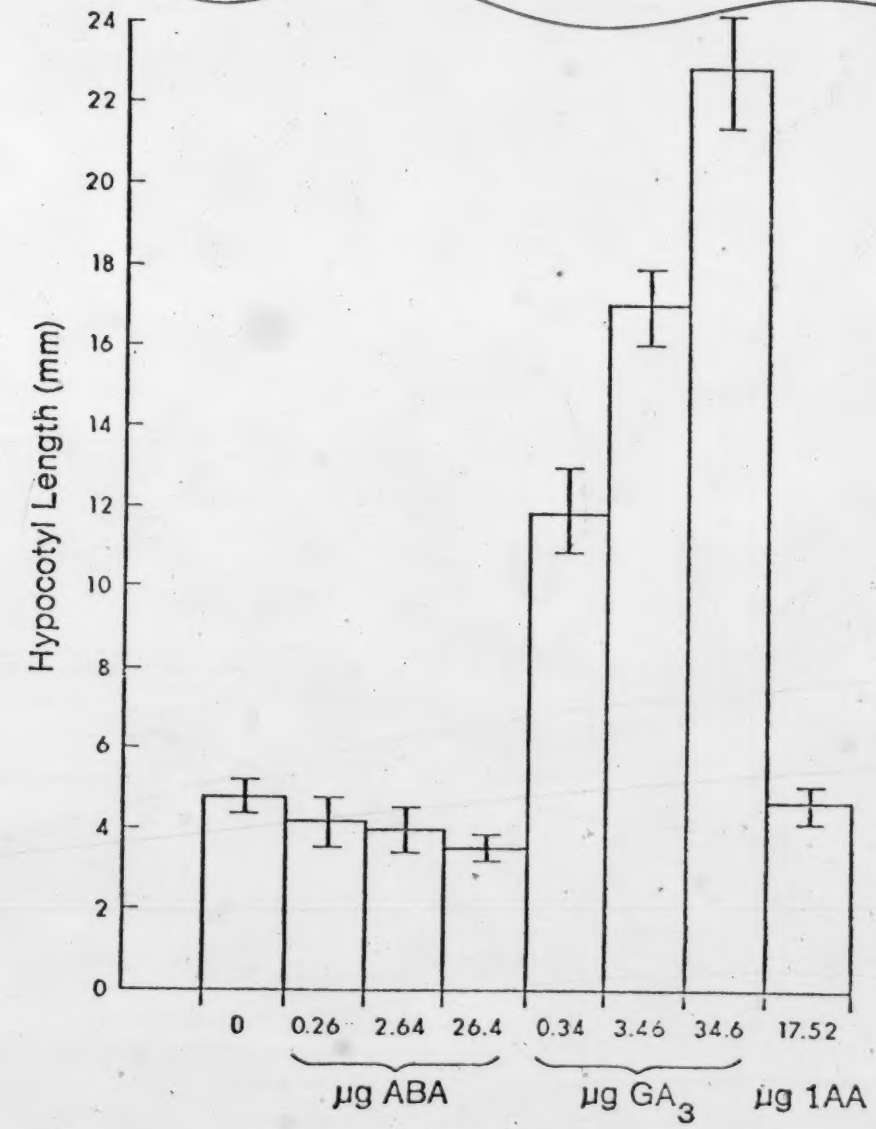

Fig. 8. Response of the lettuce hypocotyl gibberellin bioassay to concentrations of ABA, GA_3 and IAA.

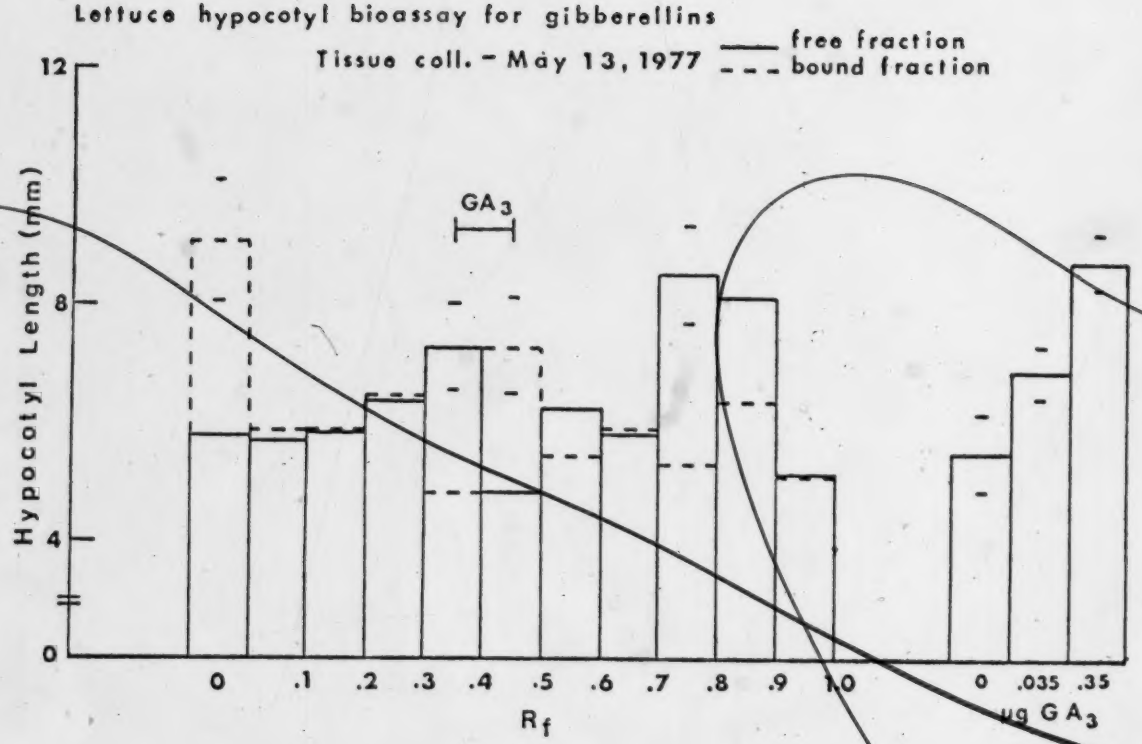

Fig. 9. Results of typical gibberllin bioassays for free and bound fractions with GA_3 standards. Confidence limits are for p = 0.05.

Soybean Callus Bioassay for Cytokinins

Soybean seeds (*Glycine max* var. Altona) were soaked in distilled water for 18 hours. In a sterile transfer room they were soaked in 5% NaOCl for 2 min. and washed twice in sterile distilled water. Each cotyledon was cut in half and the embryo discarded. Four quarter sections were placed on complete Miller's medium (Table 4), 50 mls of which was dispensed into 125-ml erlenmeyer flasks. The callus culture was conducted in the SC chamber (Table 3, Fig. 2).

Once callus tissue was produced it was used for bioassays or subcultured at monthly intervals. The bioassay consisted of transfering 4 approximately 0.05-g pieces of callus to a flask containing Miller's medium without kinetin. The test compounds were introduced by placing one of the 11 chromatogram R_f strips into a flask prior to addition of the medium. A chromatogram typically represented an extraction of 5 g fresh weight of tissue. Standards were prepared by putting known amounts of kinetin dissolved in methanol and allowing the methanol to evaporate. The test compounds, standards and controls with media were autoclaved at 121 C, 20 lbs. $\cdot$ sq in^{-1} for 15 min.. The tissue was cultured for 29 days at which time fresh weights in each flask were measured. An example of the cytokinin bioassay is shown in Fig. 10.

Exogenous Growth Regulator Experiments

These experiments were conducted with turions or plants obtained from turions. For each experiment the date of collection and the period of growth preceeding the experiment were noted. The solutions were distilled water for experiments using turions and 0.1

Table 4. Composition of Miller's medium (Miller 1967) used for culture of soybean callus tissue.

Component	Amount, mg/ℓ
NH_4NO_3	1000
KNO_3	1000
$Ca(NO_3)_2 \cdot 4H_2O$	500
$Mg\ SO_4 \cdot 7H_2O$	71.5
KH_2PO_4	300
KCl	65
(ethylenedinitrilo) tetra acetic acid sodium salt	13
$FeSO_4 \cdot 7H_2O$	9.9
H_3BO_3	1.6
$MnSO_4 \cdot 4H_2O$	14
$ZnSO_4 \cdot 7H_2O$	3.8
KI	0.8
$(NH_4)_6\ MO_7O_{24} \cdot 4H_2O$	0.1
$Cu(NO_3)_2 \cdot 3H_2O$	0.35
m-inositol	100
nicotinic acid	0.5
thiamine · HCl	0.5
α-napthalene acetic acid	2
kinetin	0.5
sucrose	30,000
Agar	10,000

pH - 5.8

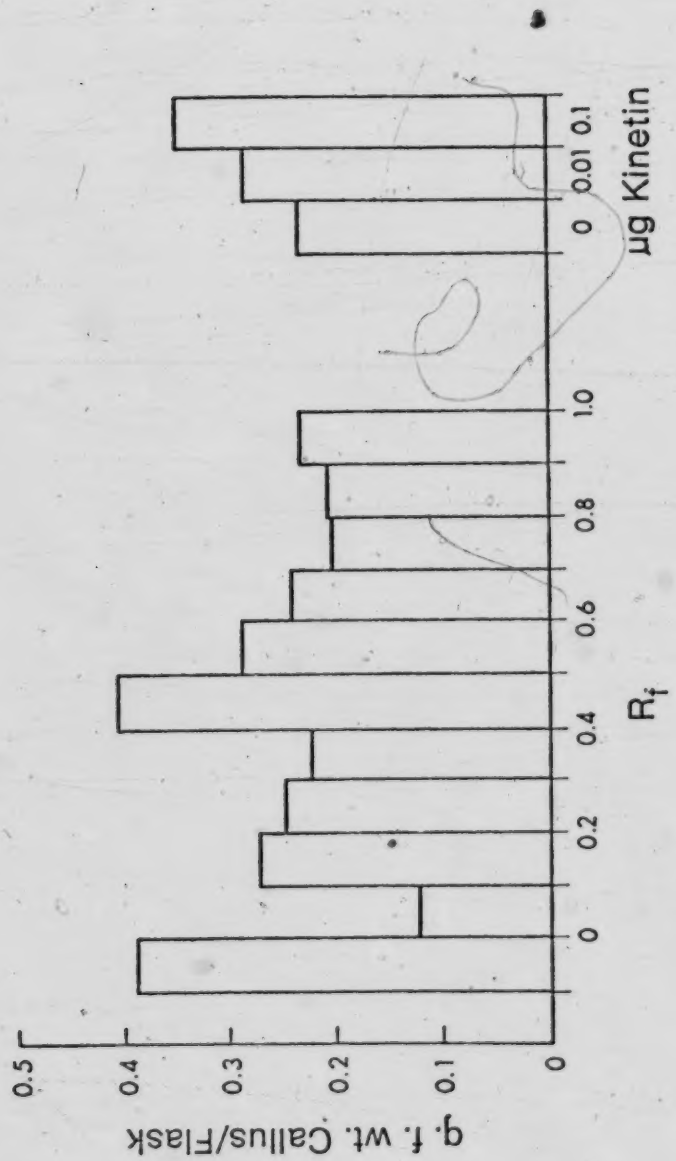

Fig. 10. Results of a typical cytokinin bioassay of tissue collected April 11, 1977 and kinetin standards.

X Hoagland's solution for experiments with actively growing plants. Solution pH was adjusted to 7-8 and solutions were not changed during the experiment unless specifically noted. Although measurements for growth (Δ fresh weight and Δ length) were made, the responses of interest were of a qualitative nature (i.e., turion induction or turion sprouting) and results were taken as the presence or absence of such a response. The controlled environment facilities used were the SD, LD and HT chambers (Table 3, Fig. 1). Synthetic growth regulators used were those described in the section on bioassays.

Starch Measurement

Tissue residue (0.4 g dry weight) from the extraction procedure was digested with 15 ml of 52% perchloric acid for 15 min at room temperature. The digest was filtered (Millipore, Type-AP, pore size-pre-filter) and the residue was washed with 10 ml of 52% perchloric acid. The combined filtrate was made to 50 ml with distilled water and 2 mls were analyzed for starch with anthrone reagent (Jermyn 1975). The anthrone reagent was prepared with 0.1 g anthrone · 100 ml^{-1} 80% (v/v) H_2SO_4. The assay consisted of adding the 2 ml test solution to 10 ml ice-cold anthrone reagent. The mixture was transfered to a boiling water bath for 100 min, cooled in an ice bath and the absorbance read with a spectrophotometer (Beckman DBG, Beckman Instruments, Fullerton, California) at 630 nm against a blank (5 ml H_2O + 5 ml 52% perchloric acid).

Measurement of Dark Respiration

The response of respiration with regard to increasing tempera-

ture was measured for various sets of plants. Apical sections (5-10 cm) were placed in a plexiglass cuvette filled with well-stirred 0.1 X Hoagland's solution. In the top of the cuvette was fixed an oxygen sensor (Beckman 39553 DO/BOD sensor) and a Cu-constantan thermocouple. The O_2 concentration was measured with an oxygen analyzer (Beckman 100800 Fieldlab) used in conjunction with a recorder (Beckman 10" recorder). Temperature control was provided by circulating water through a rubber tube coiled around the outside of the cuvette which was connected to a controlled-temperature water bath (Poly Temp model 90c, Polyscience Corp., Niles, Illinois) and by a Peltier cooling plate (Stir-Kool, model SK-12, Thermoelectrics Unlimited, Inc., Wilmington, Delaware) on which the cuvette sat.

RESULTS

Phenological Observations

Utricularia vulgaris was observed at the collection site from February to December, 1977. For this period, ambient temperature and photoperiod information was available from the Edmonton International Airport, located approximately 14 km SE of the site.

During February the plant was found in its overwintering form, the turion. The turion of *Utricularia vulgaris* is an approximately spherical apical bud composed of a tightly telescoped stem, or stems, with tightly appressed leaves. The exposed tips of the imbricate leaves have stiff, minute hairs and are tough and scale-like. These leaf tips afford protection from desiccation and mechanical damage. At this time the pond was frozen nearly to the mud and was snow-covered. Turions, less dense than water, could be found suspended by the persistent parent stem in the water column below the ice, embedded in the ice sheet and on the ice surface at the ice-snow interface. Turions were black in color and ranged in size from < 1 mm to 2.5 cm in diameter (Plate 1). Even the smallest ones collected were viable. The maximum ambient temperature in February was 11.6 C, and the minimum was -9.9 C.

By mid-March, snow cover had gone and turions at the ice surface had begun to absorb radiation during sunny periods. This initiated a daily cycle of melting and night refreezing in the ice immediately surrounding the turion (Plate 2). Those turions located just beneath the ice sheet or embedded in it also began to absorb solar radiation and melt pathways to the ice surface. After a period of these cycles the turions located in these daytime micro-pools took on a

Plate 1. Size range of turions collected April 4, 1977.

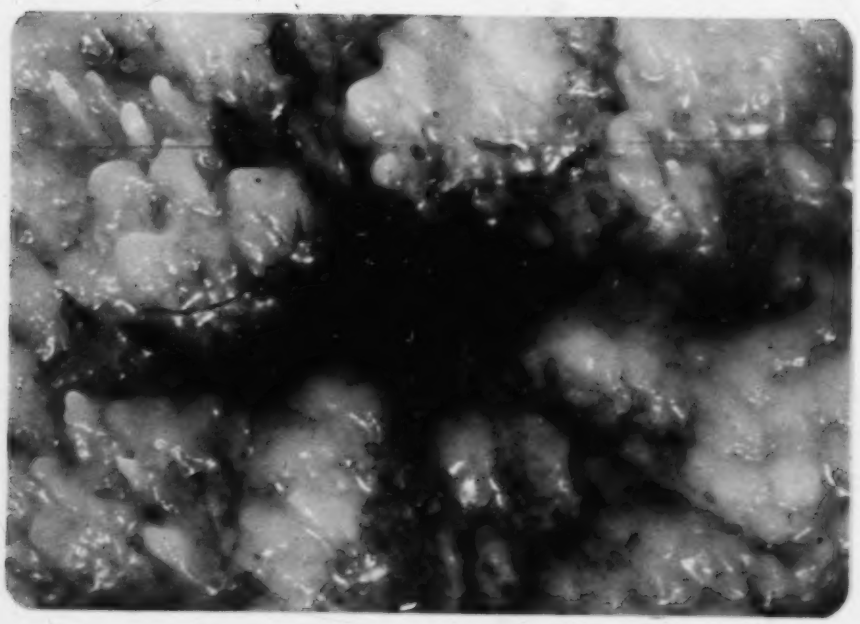

Plate 2. Example of an ice-embedded turion as seen on March 11, 1977.

green color and began to slowly reflex their leaves. The maximum air temperature during March was 11.6 C, and the minimum was -14.6 C.

Permanent ice cover was gone by April 9 at which time water temperature was measured at 16 C. This water temperature was the result of 5 previous days with ambient minimum temperatures above 0 C and a maximum of 25 C on April 8. On April 9 some turions were in the process of sprouting but were not growing rapidly. The shallow nature of the pond permitted sub-freezing ambient temperatures to cause ice cover thereby embedding the floating turions and preventing full realization of growth potential.

On May 12 the mid-afternoon water temperature was 29 C, five degrees higher than the ambient temperature. At this time *Utricularia* was in the process of rapid growth which continued throughout June. In late June and early July, flowering was initiated. Flowers were yellow, cleistogamous and deeply lobed. Typically about 10 flowers were produced on the emergent scape, and they developed acropetally. As fruit development began, the lower portion of the scape became submerged leaving the remaining flowers emergent. Fully developed fruit abscised and remained submerged. The seeds produced were viable. Flowering and fruit production continued through the third week of July.

On July 20, turion formation had begun on all plants observed. Of 40 plants collected, the average length was 63 cm, the average number of shoots per plant was 9, the average number of turions per plants was 4, 5 plants still had flowers and 19 plants were involved in fruit production. For 21 of the plants there was no evidence of flowering having taken place. At this point during the season ambient temperatures were at the summer maximum and the daylength was 43 min shorter than the

seasonal maximum (16 h and 20 min *vs* 17 h and 03 min).

Turion development continued through August. With cessation of apical growth, senescence of the older, basipetal stem began to submerge the plants. By mid-September leaves were beginning to drop off and all bladders had been abscised. The parent stem was submerged and attached turions were suspended in the water column. The turion had lost its green color and appeared dark brown. By early October all leaves had been dropped and the plant was in the form in which it would overwinter. At this time and through mid-October, the ambient minimum temperatures stayed near 0 C with ambient maximum consistently between 10-20 C. The night minimums were sufficient to cause ice cover which disappeared during the day. On October 27 the pond was covered with ice. The previous night's reported ambient minimum temperature was 0.4 C. Water temperature over the preceding 48 hours had reached 12 C (for the corresponding period the ambient maximum had reached 13.4 C) and a minimum of -1 C (with an ambient minimum temperature over the same time period of 0.4 C).

The ambient minimum temperatures for early November were consistently sub-zero and the ambient maximum temperatures were always less than 10 C. This marked the beginning of permanent ice cover. A summary of the life cycle of *Utricularia* with reference to daylength, ambient temperature, and periodic and permanent ice cover can be seen in Fig. 11. A comparison of water temperature with ambient temperatures can be seen in Table 5.

Freeze Tolerance of Turions

Turions were tested for their ability to survive sub-zero temperatures from -2 to -12 C. The results can be seen in Table 6.

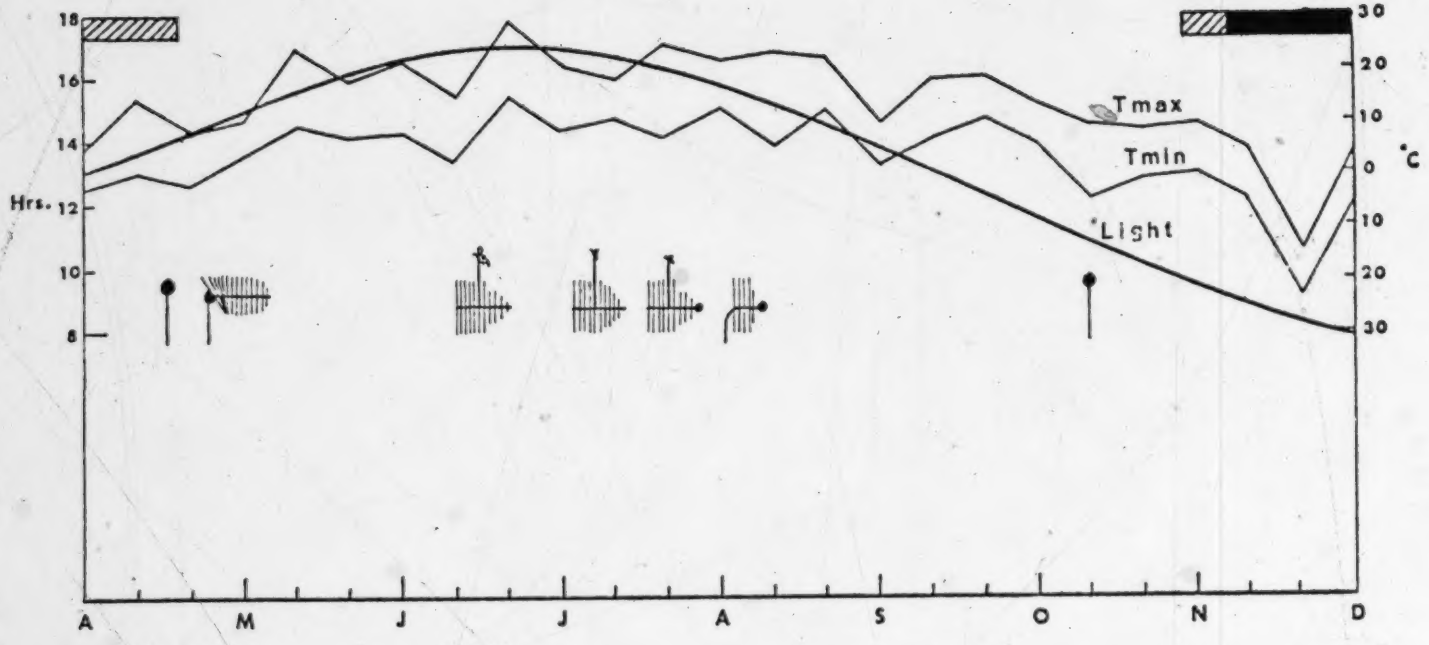

Fig. 11. Summary of environmental conditions and stage of development of *U. vulgaris* as changes occurred from April 1977 to December 1977. Development is depicted as starting with the turion, turion sprouting, flowering, fruit set and three stages of turion development. Hours of daylength, ambient temperature (max and min) and permanent ■ and episodic ▨ ice cover are shown.

Table 5. Comparison of water temperature at the collection site, measured between 12:00 - 15:00 hrs at 10 cm (and just beneath ice) with ambient max/min temperature recorded at Edmonton International Airport, 14 km away.

Date, 1977	Water Temp., °C	Ambient Temp., °C Max	Min
April 2	1	-4	-14
April 9	16	16	4
May 12	29	20	7
July 21	13	30	14
July 31	26	29	10
August 13	24	25	5
September 15	13	21	7
October 27	12	12	0.4

Table 6. Per cent survival (n=5) of turions collected and stored as indicated and subjected to sub-freezing temperatures, then tested for survival at 20°C.

Date of Collection (1977)	Storage Temp. °C	Storage Time, Days	Temps., °C -2	-5	-7	-8	-12
September 16	2-3	127	100	-	0	-	0
December 11	2-3	41	100	-	0	-	0
December 11	-5	51	100	100	-	40	0
January 31	none	0	100	100	-	100	0

Two sets of turions collected on September 16 and December 11 survived -2 C. These two sets of turions had been stored in water and refrigerated at 2-3 C since collection. Another set of tissue collected on December 11 but stored in ice as collected showed 40% survival at -8 C but no survival at -12 C. Tissue collected on January 31 and tested on that day showed complete survival at -8 C but none at -12 C.

Controlled Environment Studies

Depth of Dormancy

Turions were collected at intervals from August 6 to December 11 and immediately tested for depth of dormancy. This was done with three environmental conditions; LD (18-hour daylength, 20 C), SD (12-hour daylength, 20 C) and HT (16-hour daylength, 30 C).

The results for the LD treatment can be seen in Fig. 12a. Under these conditions tissue collected from August 13 to September 16 showed no indication of sprouting. Tissue was kept under these conditions for periods of up to 112 days without sprouting but was still viable as shown by a 30 C sprouting inductive treatment. Tissue collected on October 2 and 13 showed a minimal, delayed response after 21 and 16 days of incubation. Tissue collected on October 27 and December 11 showed a much more immediate response after 5 and 2 days incubation respectively.

Turions subjected to SD incubation (Fig. 12b) showed a similar sprouting response pattern. Tissue collected from August 13 to September 16 gave no response while that collected on October 2 and 13 exhibited a minimal and delayed response. The October 27 collection gave a much less delayed response and turions collected on December 11

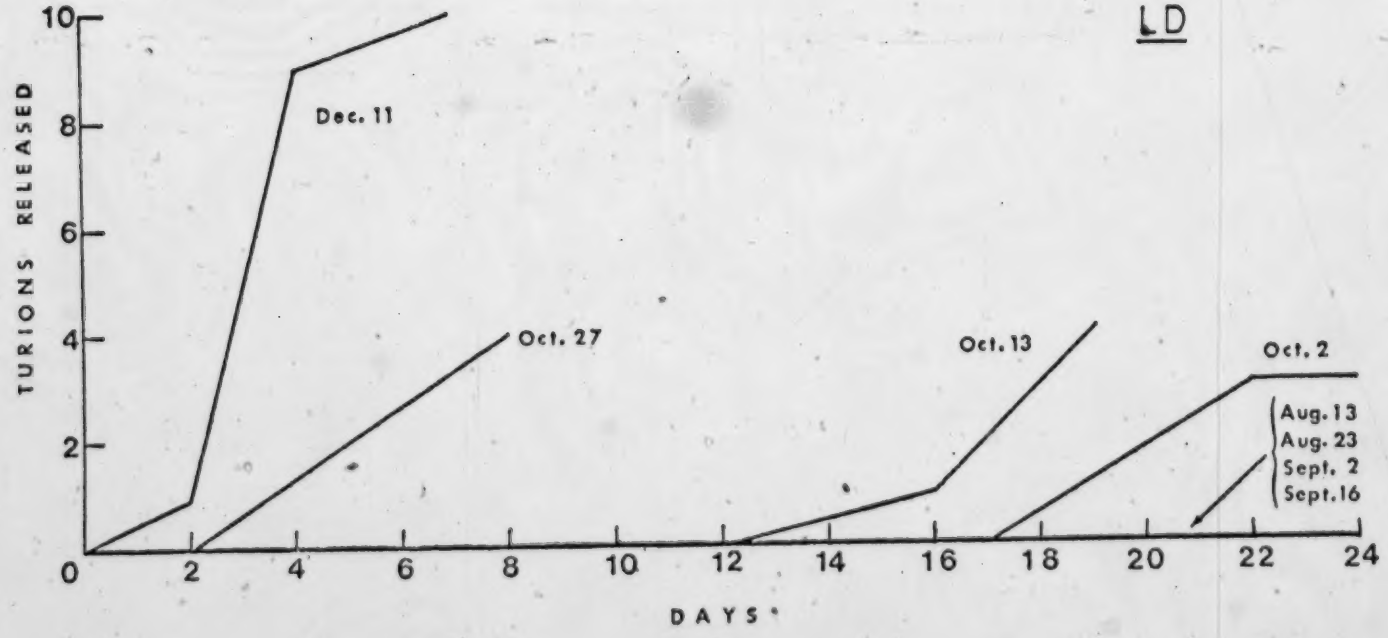

Fig. 12a. Dormancy release of ten turions collected from Aug. 13 to Dec. 11, 1977 as determined by sprouting response under LD (18-hour day, 20°C) conditions. Experiments were initiated on the date of collection.

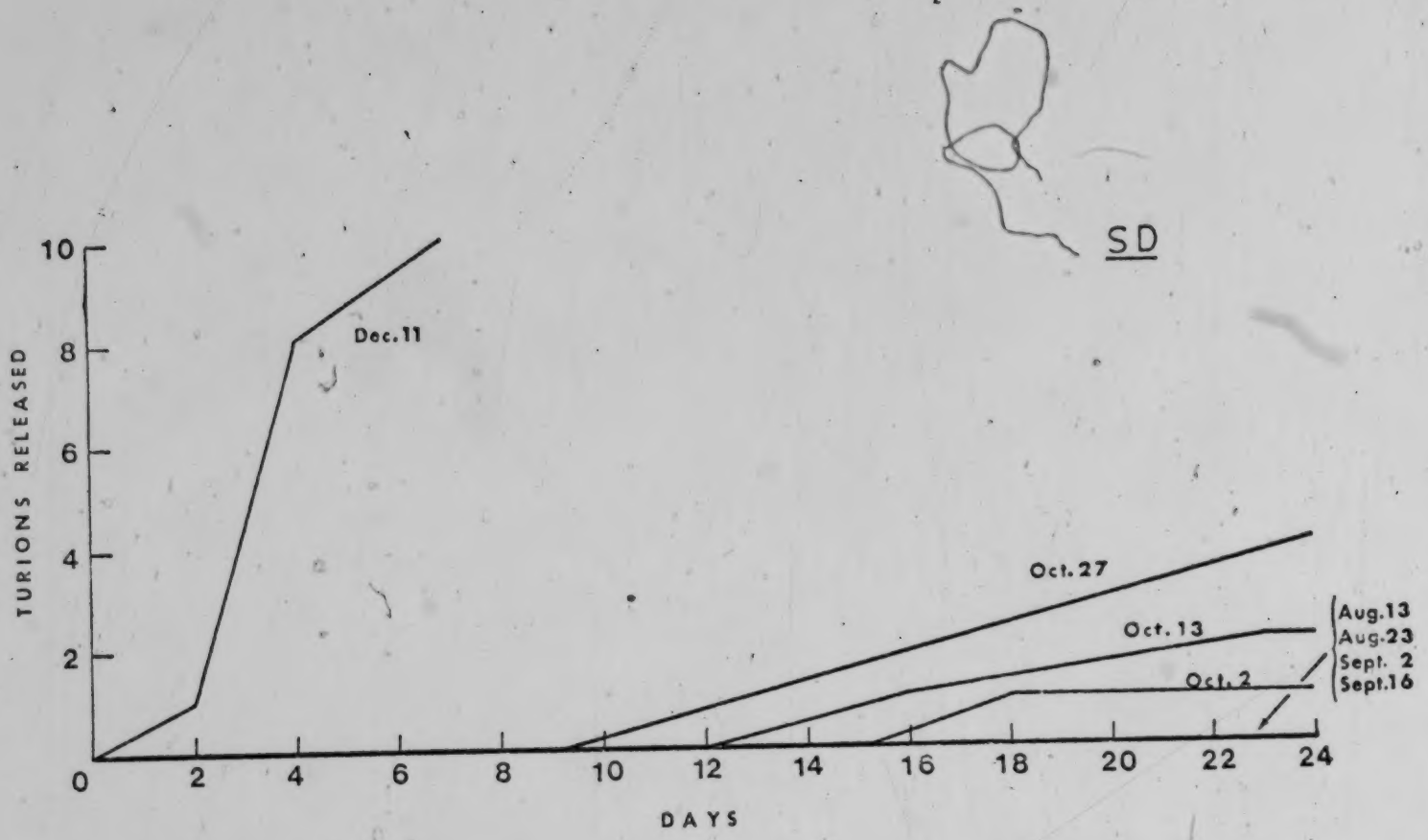

Fig. 12b. Dormancy release of ten turions collected from Aug. 13 to Dec. 11, 1977 as determined by sprouting response under SD (12-hour day, 20°C) conditions. Experiments were initiated on the date of collection.

sprouted immediately.

HT incubation elicited a sprouting response from all turions collected (Fig. 12c). Tissue collected from August 13 to September 16 exhibited a response only after a delay of 5 to 10 days. Tissue collected from October 2 to December 11 showed a complete, immediate response.

It can be seen that turions collected from August 13 to September 16 were reluctant to sprout under all conditions; not sprouting at 20 C and sprouting after a delay at 30 C. Collections from October 2 to December 11 were increasingly ready to sprout under these conditions with the 30 C treatment enhancing the response obtained at 20 C. These patterns are summarized in Fig. 13. This figure shows that the date at which the sprouting response at 20 C and 30 C changed significantly was just prior to October 1. Fig. 13 also points out the finding that LD treatment slightly enhanced the rate of sprouting when compared to the SD treatment.

Although depth of dormancy was determined primarily in this study by the turion sprouting response in favorable growth conditions this is not necessarily a definitive indicator. It was observed that turions caused to sprout by HT treatment or by artificial chilling and then grown at 20 C (LD or SD) would elongate for no more than three weeks at which time a turion would be reformed (Table 7). Plants from turions collected prior to September 16 formed turions regardless of daylength. Those from turions collected after October 2 did not form turions regardless of daylength. Plants from the intermediate collections of September 16 and October 2 were susceptible to daylength and responded to SD incubation by forming turions but did not form turions under LD conditions.

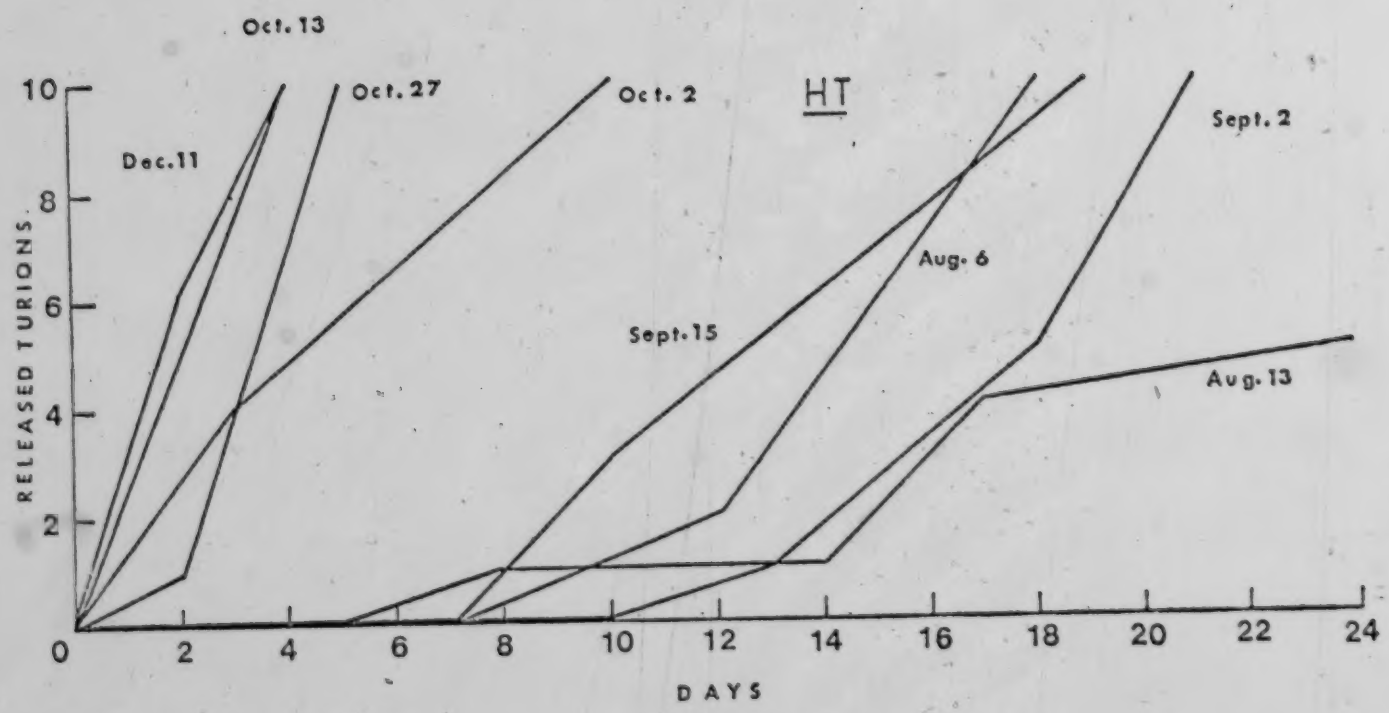

Fig. 12c. Dormancy release of ten turions collected from Aug. 6 to Dec. 11, 1977 as determined by sprouting response under HT (16-hour day, 30°C) conditions. Experiments were initiated on date of collection.

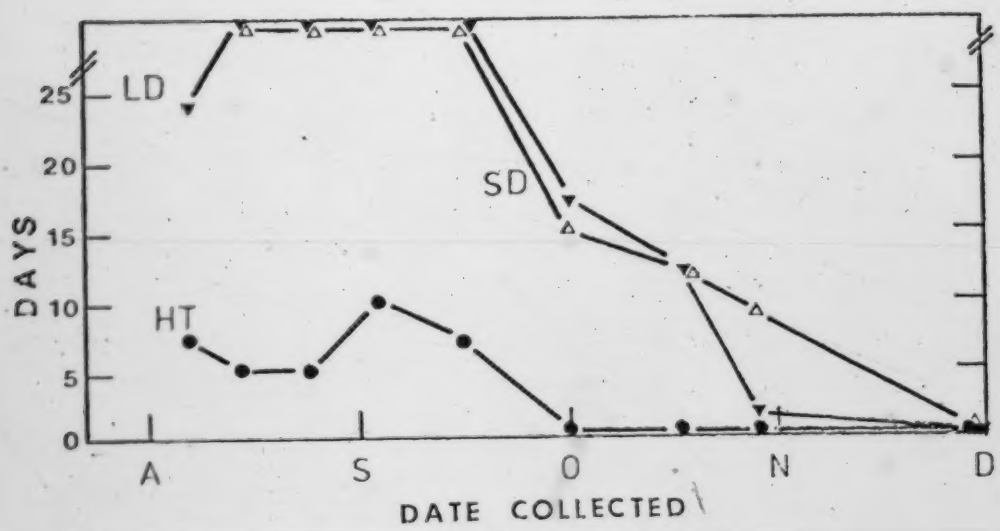

Fig. 13. Summary of depth of dormancy experiments. Days required to get initiation of the sprouting response for tissue collected between April and December, 1977.

Table 7. Turion reformation by plants collected from July 21 to October 13, 1977. Plants were scored for presence (+) or absence (-) of turions within three weeks of experiment initiation.

Experiment Date	Collection Date	Growth Chamber LD	Growth Chamber SD
July 21	July 21	+	
August 9	July 21	+	
August 20	August 6		+
August 20	August 13	+	
August 20	August 13	+	
October 30	August 23	+	
October 30	September 2	+	
October 30	September 16	-	
October 30	October 2	-	
October 30	October 13	-	
November 11	September 2	+	
November 11	September 16	-	
November 11	October 2	-	+
November 11	October 13	-	-

Another correlation with the loss of potential to re-enter dormancy was an observable difference in rate of sprouting. This was manifested by much more rapid elongation such that a single turion would completely fill a 125 ml flask within 3 days. It was also observed that this sprouted turion released much less mucilage in the flask than did the sprouted turions collected earlier in the season. Finally, sprouted turions collected prior to October 2 produced fewer air shoots than did those collected later.

Effect of Artificial Chilling

Turions collected during the study period were refrigerated at 2-3 C and samples were taken from these and tested for depth of dormancy. Examples of these experiments are given for collections on July 21 (Figs. 14a,b), August 23 (Figs. 15a,b,c), September 2 (Figs. 16a,b,c), September 16 (Figs. 17a,b,c) and October 2 (Figs. 18a,b,c).

The turions collected on July 21 were the first formed and were in very limited supply. They were tested for sprouting (as were all other collections) after being separated from the remainder of the plants. Under LD conditions (Fig. 14a) there was a delayed sprouting response while under HT conditions (Fig. 14b) there was an immediate response with 3 days of chilling causing a slight delay.

Turions collected on August 23 and incubated in the SD chamber (Fig. 15a) showed no response when tested after 0 and 12 days of chilling. Twenty-four days of chilling resulted in a minimal response while 40 and 51 days at 2-3 C gave a much more complete sprouting response. This pattern is repeated in the LD experiments (Fig. 15b). Zero and 12 days of chilling gave no response, 24 days resulted in a minimal response and 40, 51 and 110 days resulted in nearly complete and

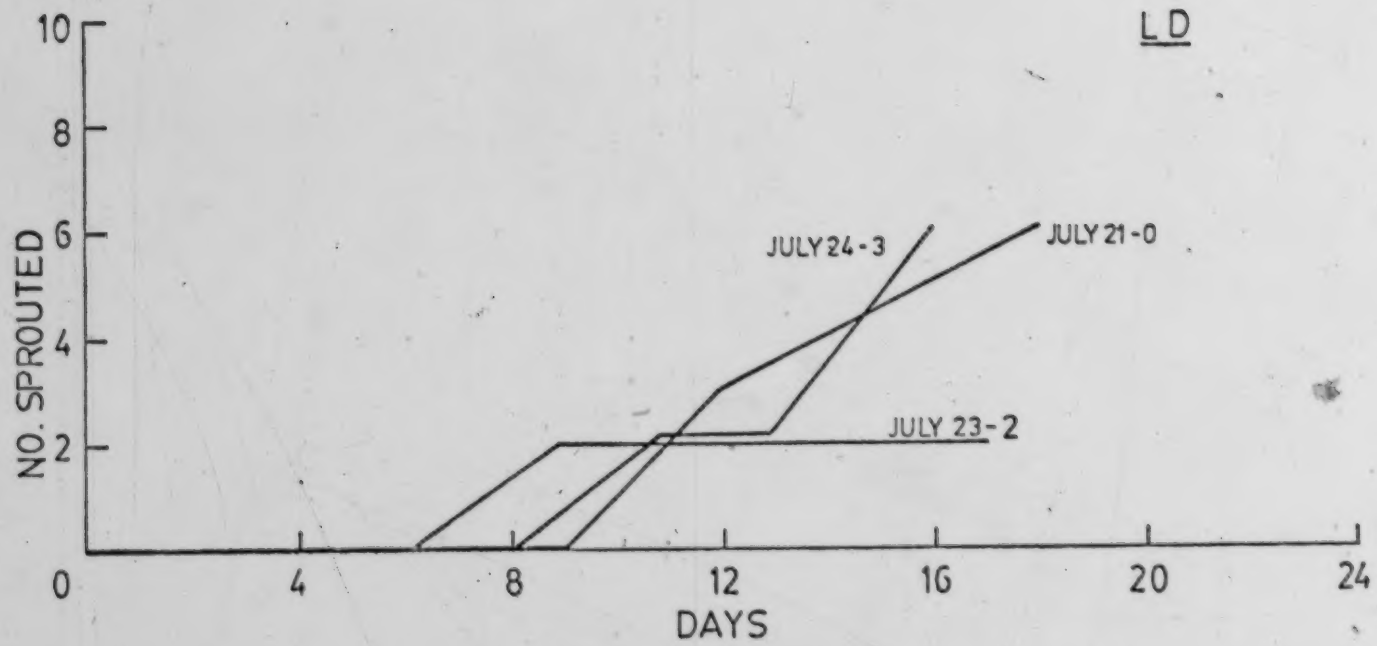

Fig. 14a. Sprouting response of 10 turions collected on July 21, 1977 to varying periods of artificial chilling and tested under LD conditions on the dates indicated. Days of chilling are given after test dates.

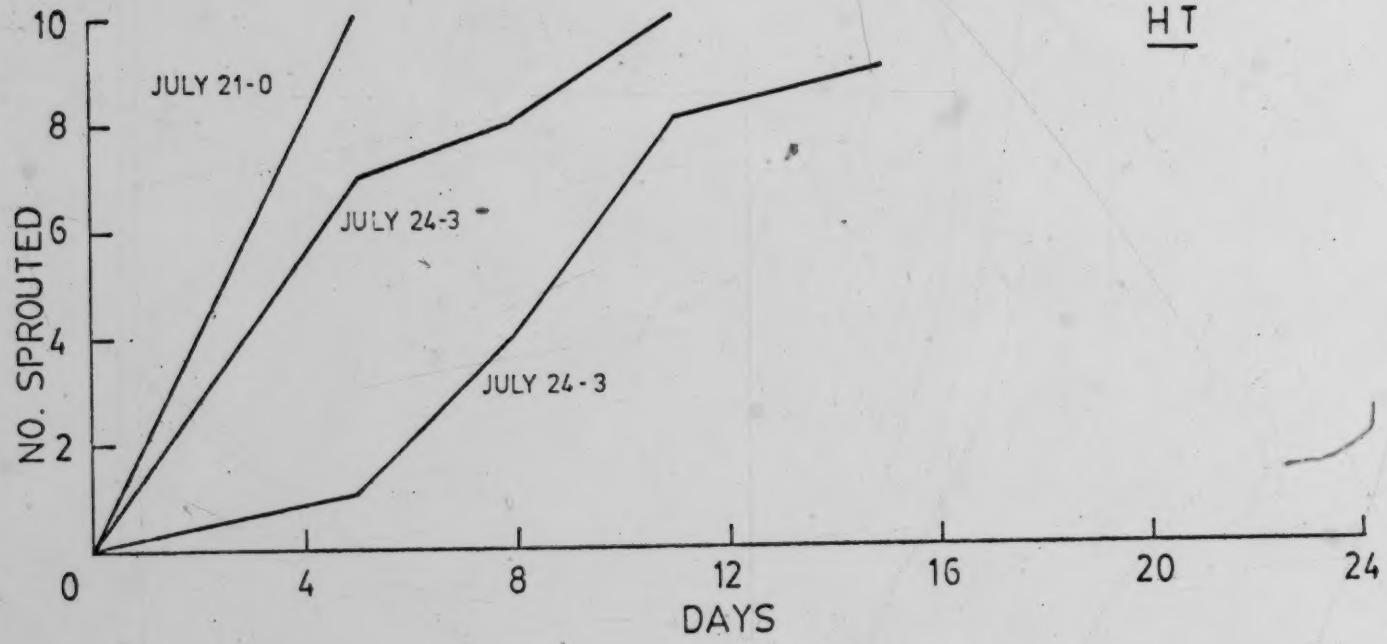

Fig. 14b. Sprouting response of 10 turions collected on July 21, 1977 to varying periods of artificial chilling and tested under HT conditions on the dates indicated. Days of chilling are given after test dates.

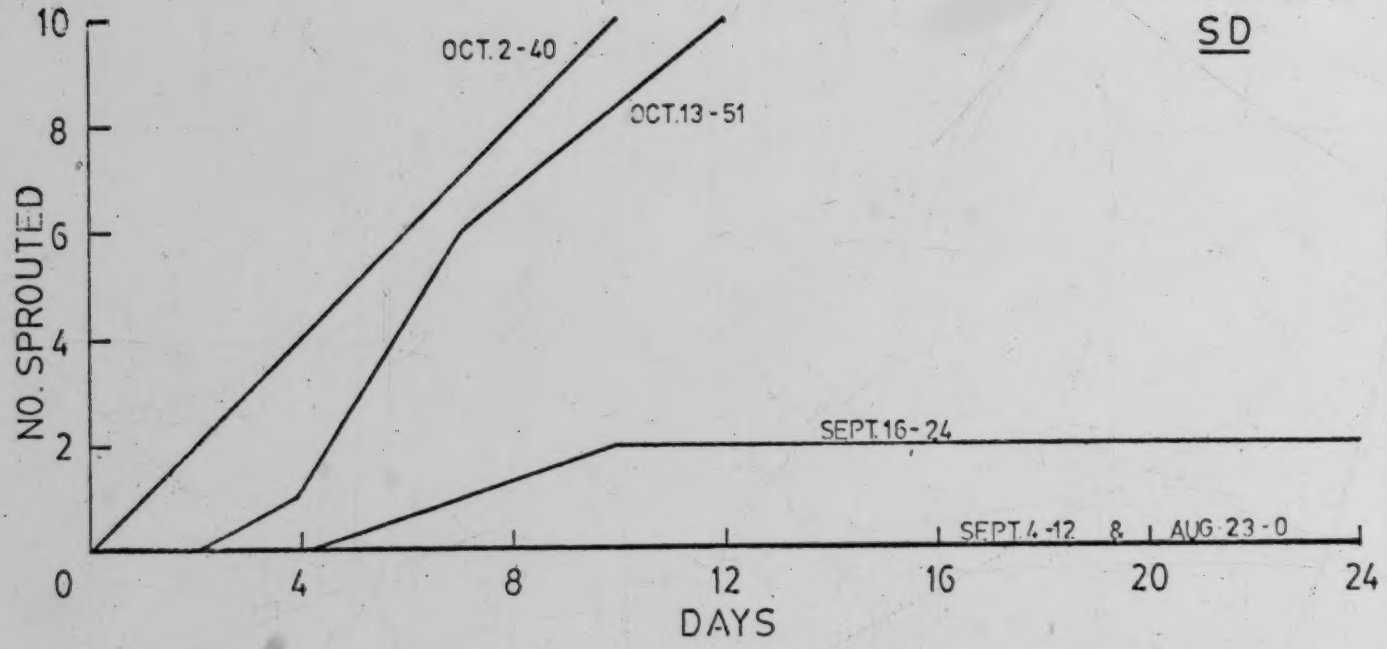

Fig. 15a. Sprouting response of 10 turions collected on Aug. 23, 1977 to varying periods of artificial chilling and tested under SD conditions on the dates indicated. Days of chilling are given after test dates.

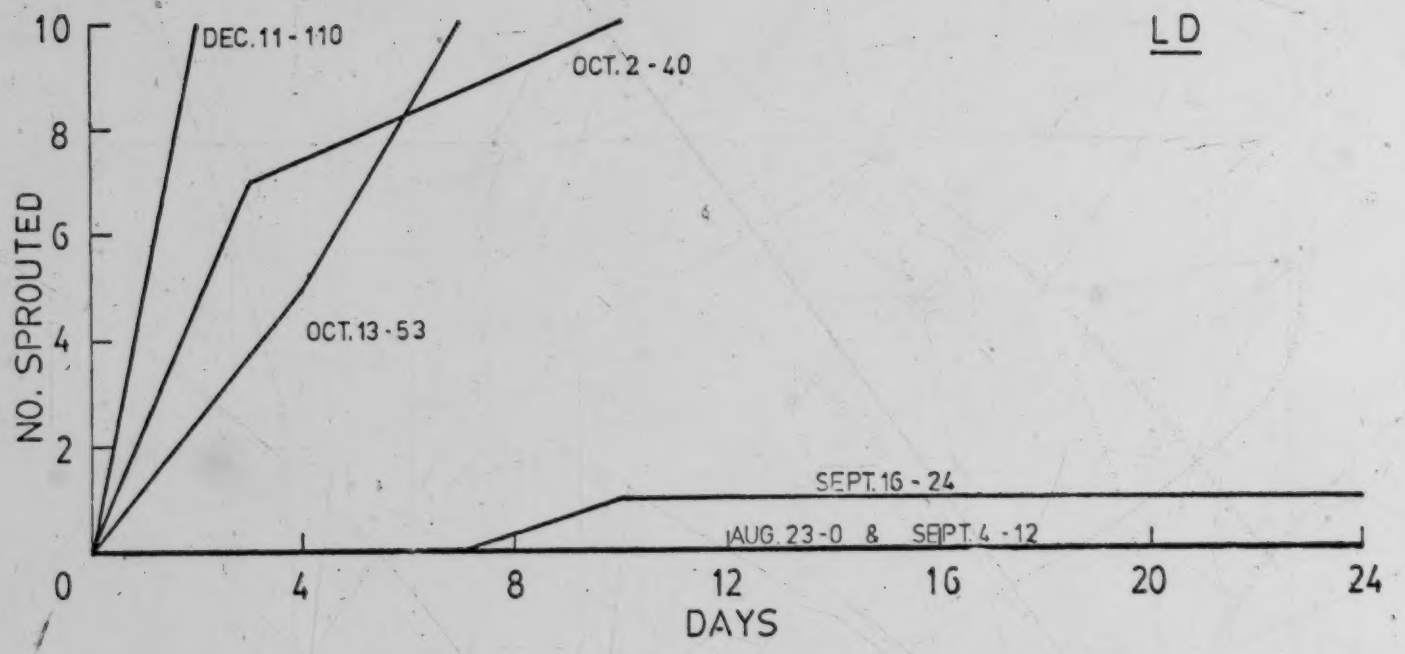

Fig. 15b. Sprouting response of 10 turions collected on Aug. 23, 1977 to varying periods of artificial chilling and tested under LD conditions on the dates indicated. Days of chilling are given after test dates.

immediate responses. Under HT conditions (Fig. 15c), 0 and 12 days of chilling resulted in a lag response while 24, 40 and 65 days at 2-3 C produced an immediate response.

For turions collected on September 2, 0 and 2 days of chilling resulted in no response in the SD chamber (Fig. 16a). After 14 days at 2-3 C a delayed, minimal response was observed. Chilling for 30 days produced a slightly greater response and 41 and 55 days produced a much faster and more complete response. The response to LD conditions (Fig. 16b) followed a similar pattern but one which much more clearly separated the two periods of chilling in terms of sprouting response; 0, 2 and 14 days at 2-3 C resulted in no response whereas 30, 41, 55 and 100 days produced increasingly rapid and complete responses. This separation of the two periods of chilling in terms of sprouting response is also apparent for turions collected on September 2 in tests done with HT conditions (Fig. 16c). Here 0, 2 and 14 days produced a lag response while that tissue chilled for 30, 41 and 55 days exhibited an immediate and nearly complete response.

Turions collected on September 16 and subjected to SD conditions (Fig. 17a) did not sprout unless chilled. A minimal response was achieved with chilling periods of 16 to 27 days while periods of 41 to 85 days showed a much greater response. The response of these turions to LD conditions (Fig. 17b) produced a similar pattern, with no chilling resulting in no response, 16 and 27 days producing a minimal response and 41 and 85 days at 2-3 C eliciting a rapid response. This pattern was followed by those turions incubated in the HT (Fig. 17c) chamber in that 0 chilling produced a lag response while 17, 28 and 41 days at 2-3 C produced immediate sprouting.

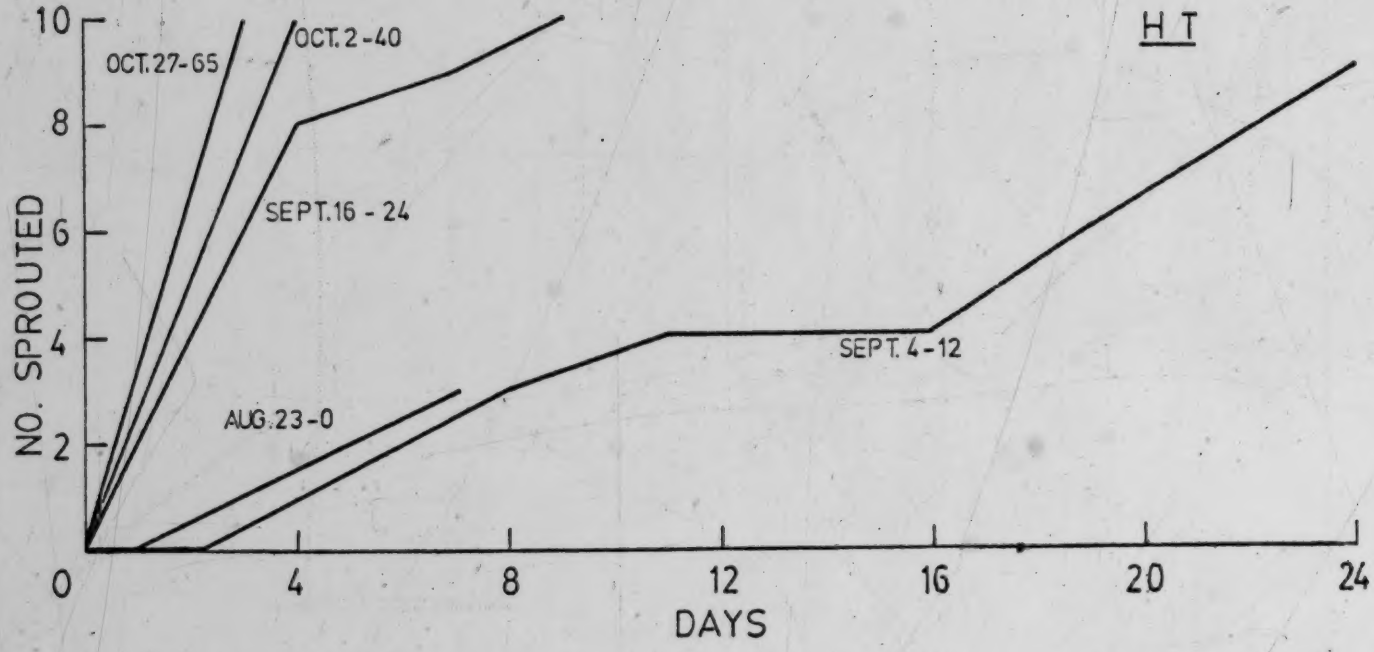

Fig. 15c. Sprouting response of 10 turions collected on Aug. 23, 1977 to varying periods of artificial chilling and tested under HT conditions on the dates indicated. Days of chilling are given after test dates.

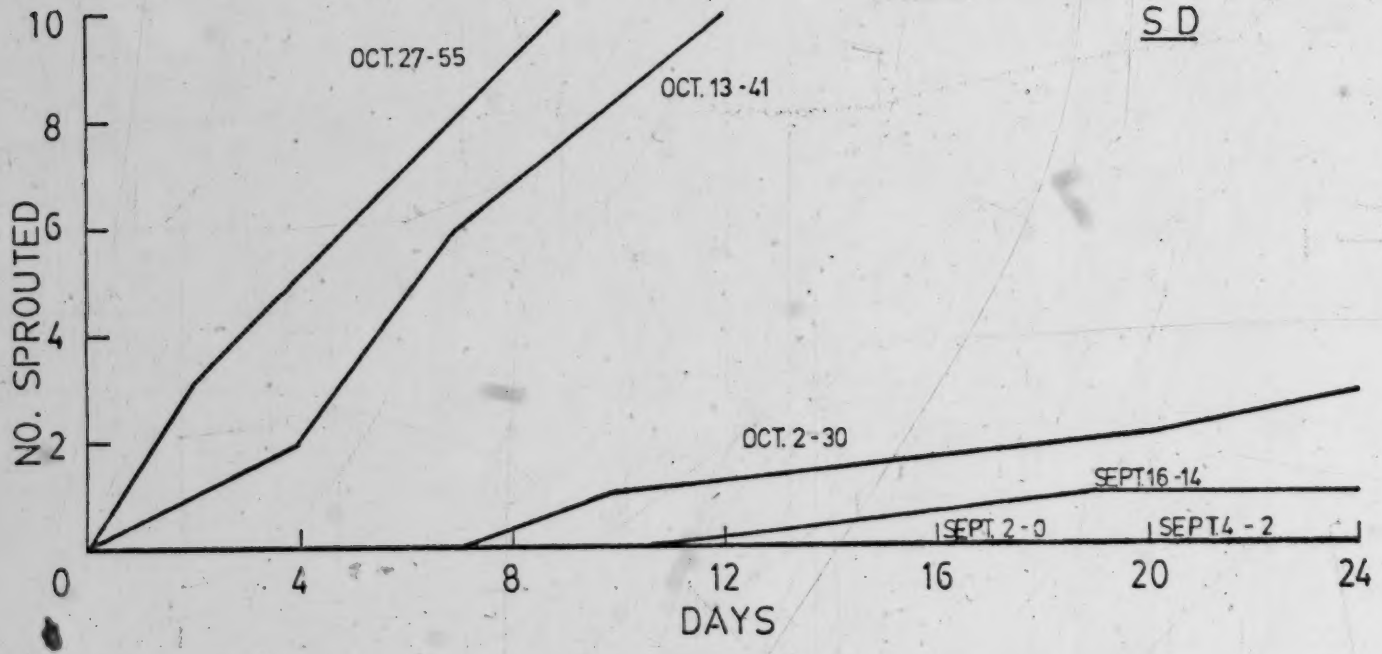

Fig. 16a. Sprouting response of 10 turions collected on Sept. 2, 1977 to varying periods of artificial chilling and tested under SD conditions on the dates indicated. Days of chilling are given after test dates.

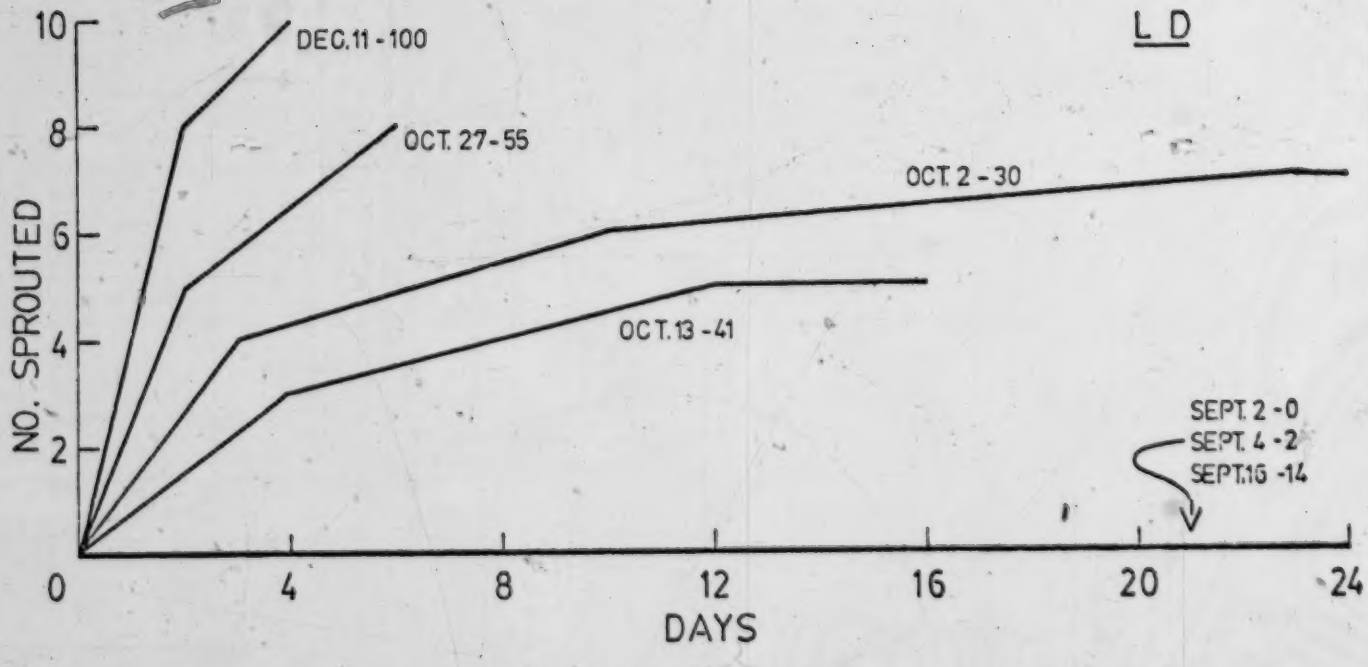

Fig. 16b. Sprouting response of 10 turions collected on Sept. 2, 1977 to varying periods of artificial chilling and tested under LD conditions on the dates indicated. Days of chilling are given after test dates.

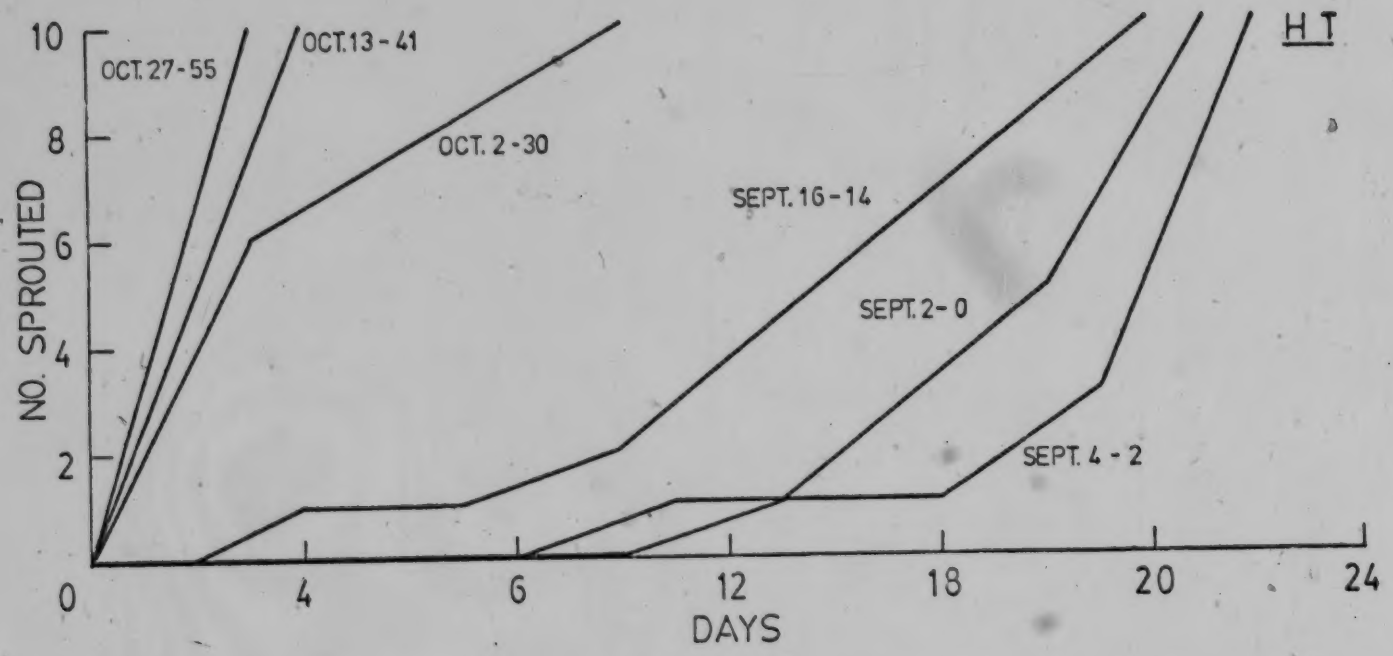

Fig. 16c. Sprouting response of 10 turions collected on Sept. 2, 1977 to varying periods of artificial chilling and tested under HT conditions on the dates indicated. Days of chilling are given after test dates.

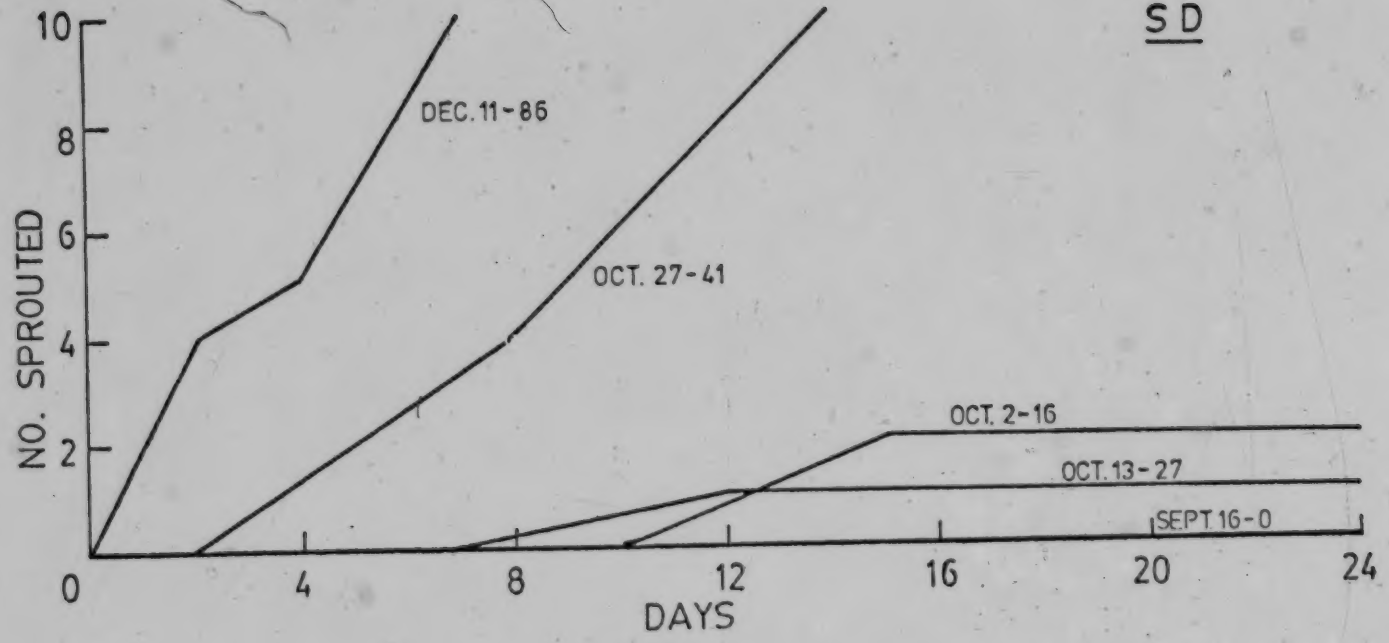

Fig. 17a. Sprouting response of 10 turions collected on Sept. 16, 1977 to varying periods of artificial chilling and tested under SD conditions on the dates indicated. Days of chilling are given after test dates.

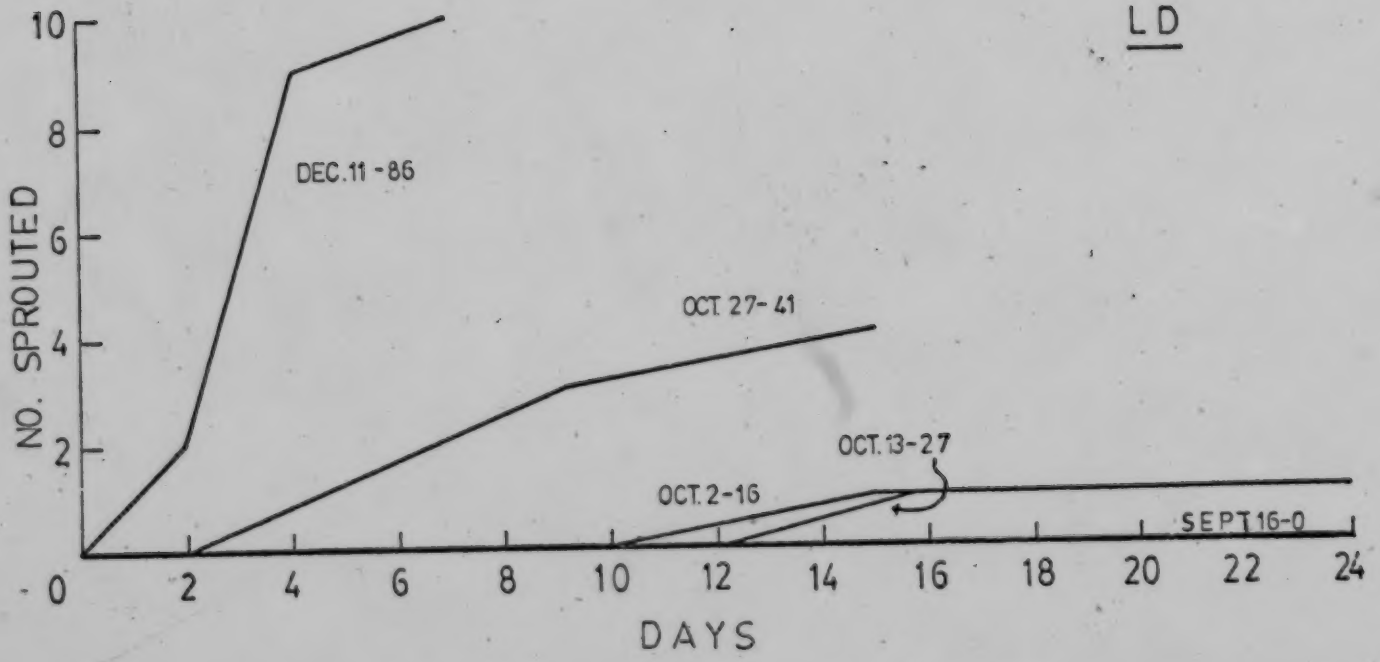

Fig. 17b. Sprouting response of 10 turions collected on Sept. 16, 1977 to varying periods of artificial chilling and tested under LD conditions on the dates indicated. Days of chilling are given after test dates.

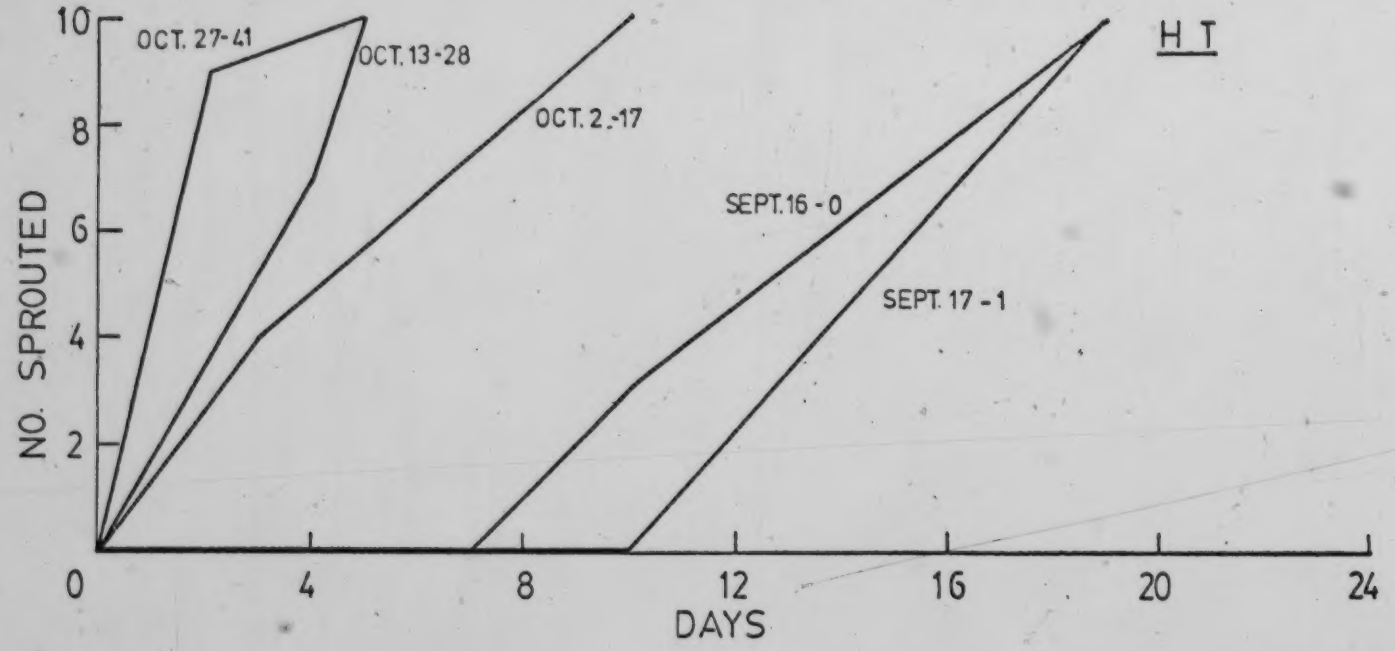

Fig. 17c. Sprouting response of 10 turions collected on Sept. 16, 1977 to varying periods of artificial chilling and tested under HT conditions on the dates indicated. Days of chilling are given after test date.

Turions collected on October 2 and incubated in the SD chamber (Fig. 18a) exhibited a minimal and delayed response if not pre-chilled. The sprouting response increased in rate with increasing periods of chilling of 11, 25 and 70 days. LD conditions (Fig. 18b) produced a similar pattern of increasing response with longer periods of chilling. HT treatment (Fig. 18c) caused rapid sprouting with no chilling and even more rapid responses with 11 and 25 days of chilling.

In terms of an immediate sprouting response in HT conditions and an initial response in the SD and LD environments the data is summarized in Fig. 19. It can be seen that to obtain a response in SD and LD conditions chilling periods of about 10 days were required for turions collected in early August. This chilling requirement increased dramatically to 30-35 days for turions collected in late August and early September. Turions collected on October 1 had a reduced chilling requirement of about 17 days; intermediate to the requirements of earlier and later collections. The collections on and after October 13 required no chilling. The immediate response obtained in HT conditions closely followed this pattern of chilling requirements with short periods at 2-3 C required in early August, longer chilling periods in late August and September and no chilling requirement for tissue collected on or after October 2.

Effect of Light on Dormancy

In order to see if light was directly involved in the sprouting response of turions placed in forcing conditions (HT), dark incubation and a brief red light treatment followed by dark incubation were compared to normal growth chamber light. As shown in Table 8, after two days of incubation at 30 C the turions

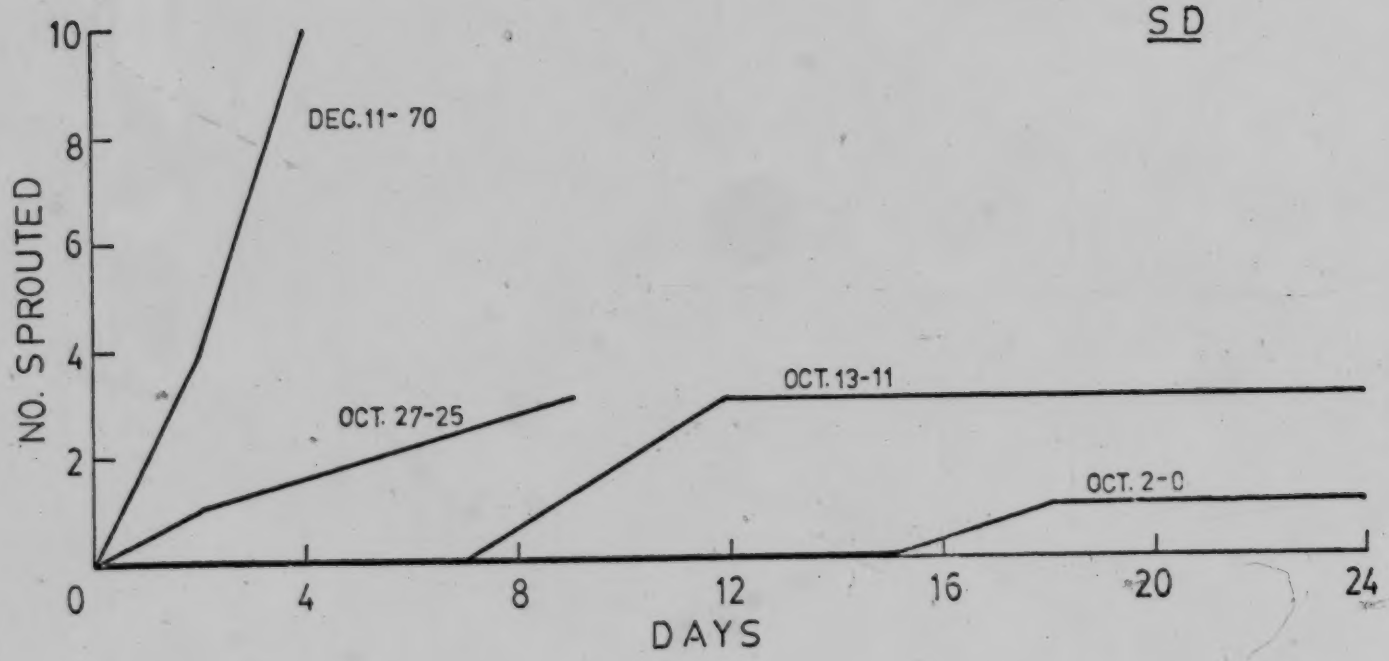

Fig. 18a. Sprouting response of 10 turions collected on Oct. 2, 1977 to varying periods of artificial chilling and tested under SD conditions on the dates indicated. Days of chilling are given after test dates.

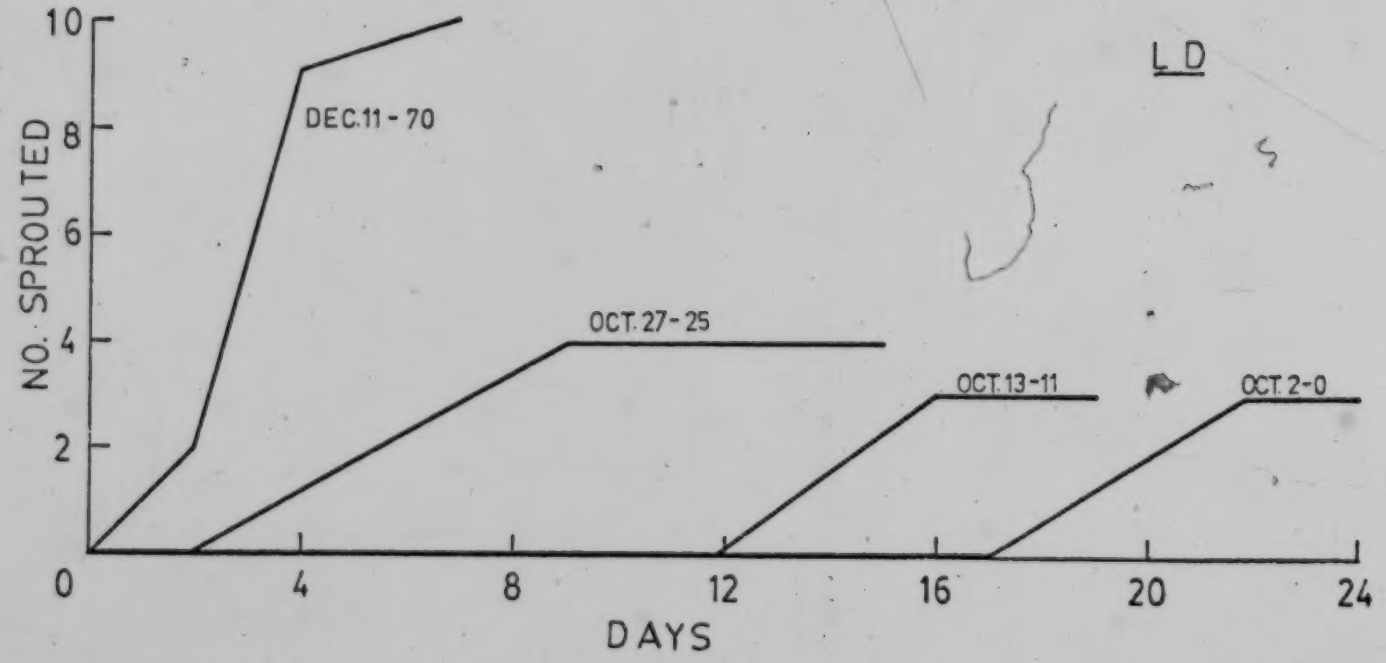

Fig. 18b. Sprouting response of 10 turions collected on Oct. 2, 1977 to varying periods of artificial chilling and tested under LD conditions on the dates indicated. Days of chilling are given after test dates.

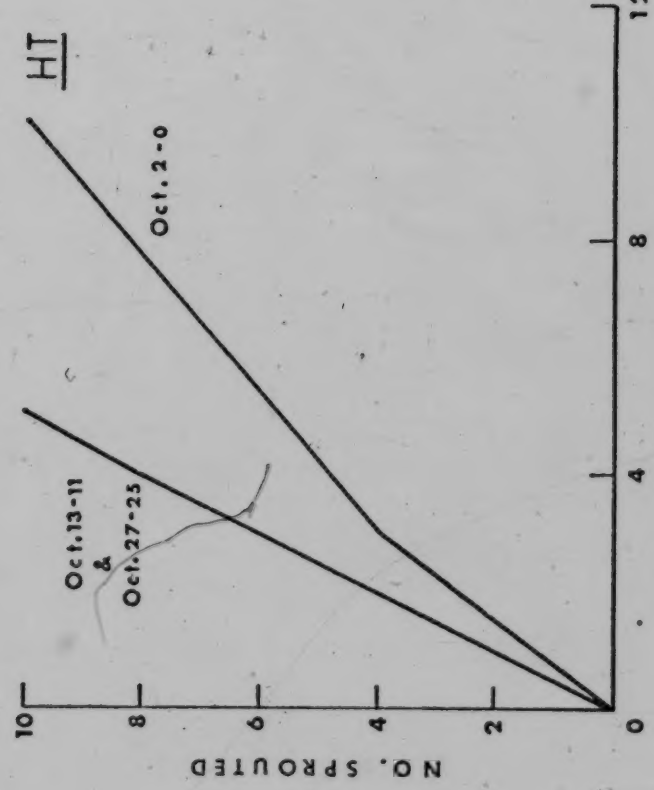

Fig. 18c. Sprouting response of 10 turions collected on Oct. 2, 1977 to varying periods of artificial chilling and tested under HT conditions on the dates indicated. Days of chilling are given after test dates.

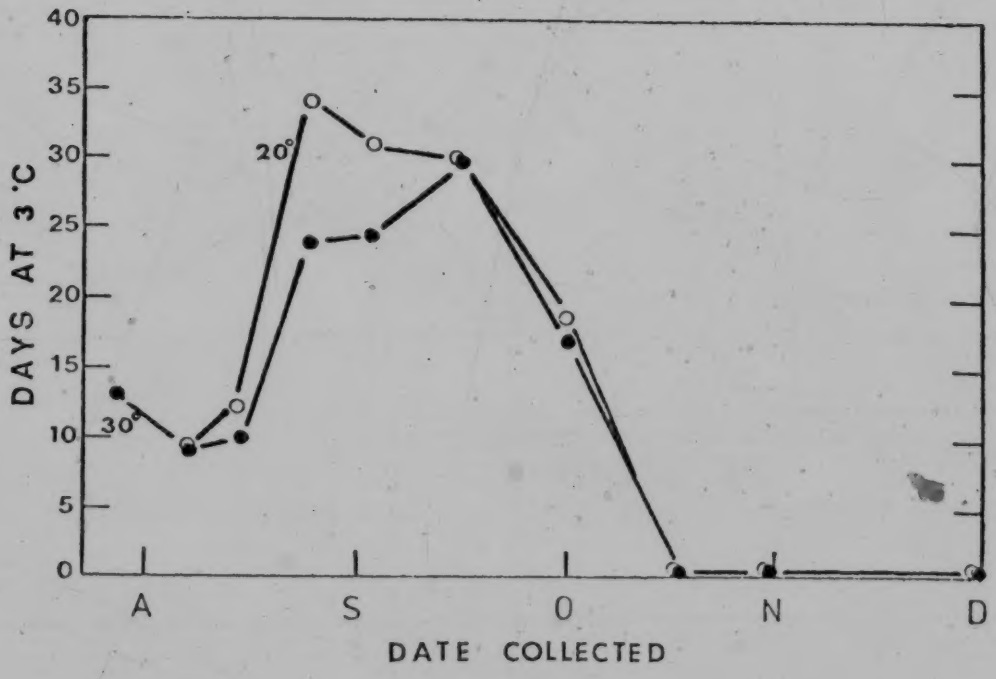

Fig. 19. Chilling time required for various collections of turions (from July 28 to Dec. 1, 1977) to obtain an immediate sprouting response at 30°C (HT) or an initial sprouting response at 20°C (SD and LD).

Table 8. The sprouting response to various light treatments at high temperature (30°C) of lots of 20 turions collected August 13, 1977 and chilled for 45 days.

Lights Treatments	Number sprouted after 2 days	4 days	7 days
High	16	20	20
Dark	0	3	20
Red (15 min.) Dark	0	6	20

exposed to chamber light were obviously sprouting while the other two sets of turions were not. This lag response to the dark and red light treatments required 7 days to overcome.

Experiments Related to Dormancy Induction

Preliminary experiments were conducted in order to investigate the possibility of a photoperiod - temperature involvement in turion induction. The results of two experiments conducted are shown in Table 9. In the experiment conducted for a period of 36 days both 15 C and 20 C treatments under SD conditions resulted in turion formation at the apex of each main axis while no plants grown under LD conditions produced turions. In the experiment which was terminated after 22 days only plants at 15 C under SD conditions produced turions. Enhanced lateral branch formation occurred in both the 15 C and 20 C, SD treatments but not under a 20 C, LD treatment.

Aspects of Dormancy Control by Plant Growth Regulators

Levels of ABA as Detected by Gas-Liquid Chromatography

Results from this work were inconclusive. GLC of plant extracts under the prescribed conditions gave a broad peak co-chromatographing with ABA. This indicated that unresolved compounds were interfering. A GLC trace accompanied by a standard ABA trace is shown in Fig. 20.

Levels of Growth Regulators Determined by Bioassay

Levels of Auxins

Auxin activity was detected at 2 R_f zones; 0.3-0.4 and 0.6-0.8.

Table 9. Turion reformation by plants (n=3) from turions collected October 27, 1977 maintained under LD conditions for 9-14 days and then subjected to various environmental conditions (plus one growth substance variable).

Previous growth period, days, under LD conditions	Incubation conditions	Period, days	Turions
9	SD	36	+
9	LD	36	-
9	SD	36	+
9	LD	36	-
14	SD[1]	22	+
14	SD	22	-
14	LD	22	-
14	SD[2]	22	+

[1] Temperature 15°C

[2] Temperature 15°C and culture solution contained 1 X 10^{-5} M kinetin.

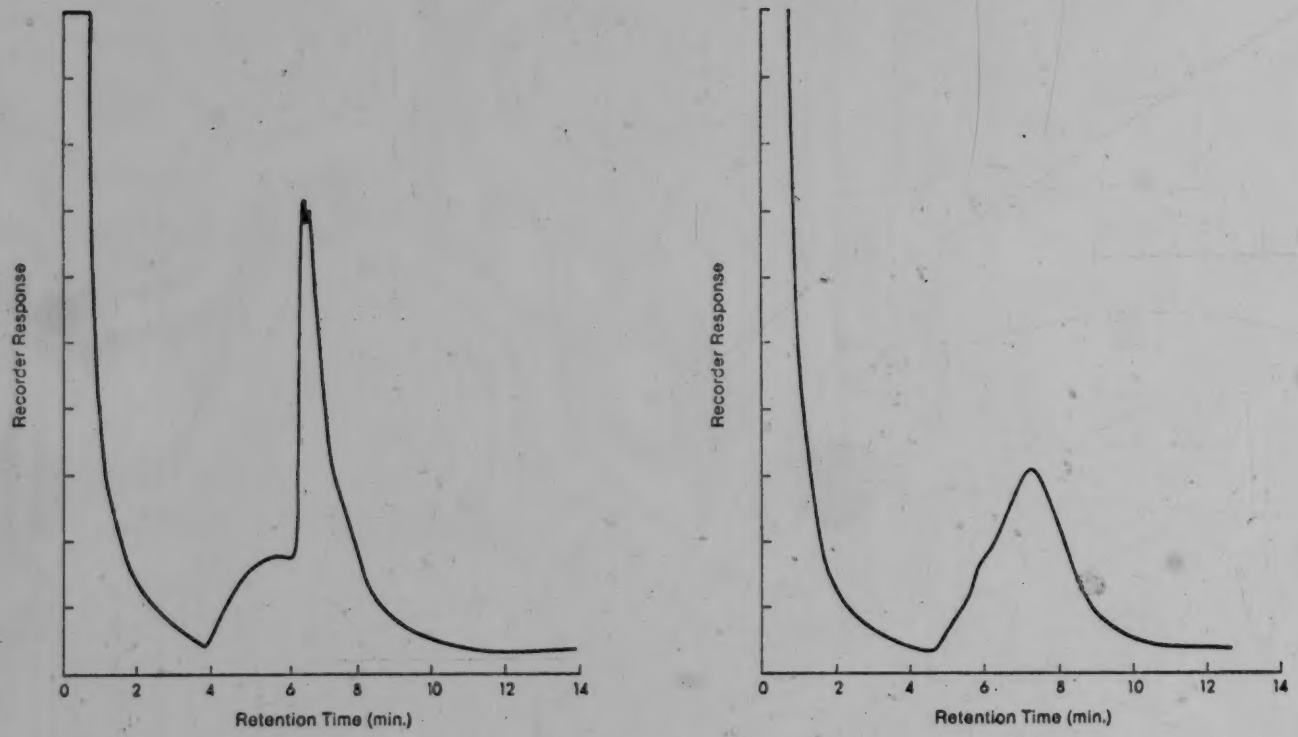

Fig. 20. Gas chromatograms obtained from the injection of 3 µl of silylated ABA standard (left) and an acidic ether extract (right) of *Utricularia vulgaris* collected on September 15, 1977.

Auxin activity at R_f 0.3-0.4, along with relative levels of the bioassay inhibitor co-chromatographing, is shown in Fig. 21. It should be noted that the bioassay inhibitor was always detected in turion tissue and was found in highest concentrations in turions collected from July 28 to September 1. In late March auxin-like activity found at R_f 0.3-0.4 was high but fell in mid-April. Bound components of this auxin-like compound were present in mid-April. Levels in mid-May were very high with the bound component barely detectable. Tissue collected during the first week of July had very low levels of both free and bound components of this auxin-like substance. Detected activity remained low throughout the remainder of the sampling period. Artificial chilling of turions collected on August 23 for 40 days resulted in higher activity; possibly due to a reduction of the co-chromatographing inhibitor.

Auxin-like activity at R_f 0.6-0.8 followed a nearly opposite pattern (Fig. 22). Activity in late March was very high and fell sharply in mid-April. Bound components of this auxin-like substance were measurable at this time. The mid-May sample had low levels of the free component but higher activity of the bound component. Both bound and free levels of this auxin were very low in early July. From this point on the free level increased to reach a maximum in the sample collected on August 23. Tissue collected on October 27 had a lower level of this auxin-like substance.

Levels of Inhibitors

There was inhibitory activity detected at two R_f zones; R_f 0.2-0.4 and R_f 0.5-0.7.

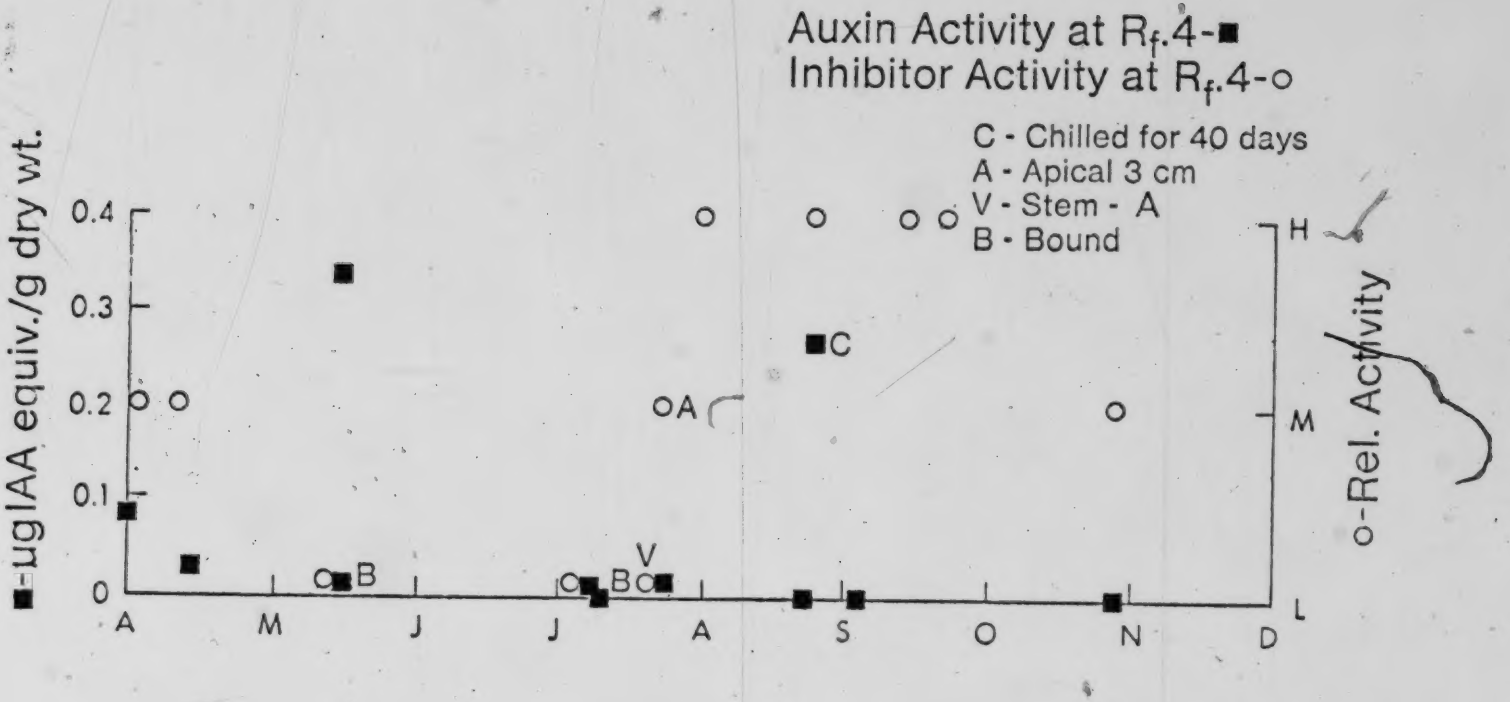

Fig. 21. Relative levels of auxin-like activity (R_f 0.4) detected in extracts of *U. vulgaris* tissue collected during 1977. Relative activity of a co-chromatographing bioassay inhibitor are also shown. Samples collected or treated differently are indicated by the appropriate code letters (see Discussion and Conclusions).

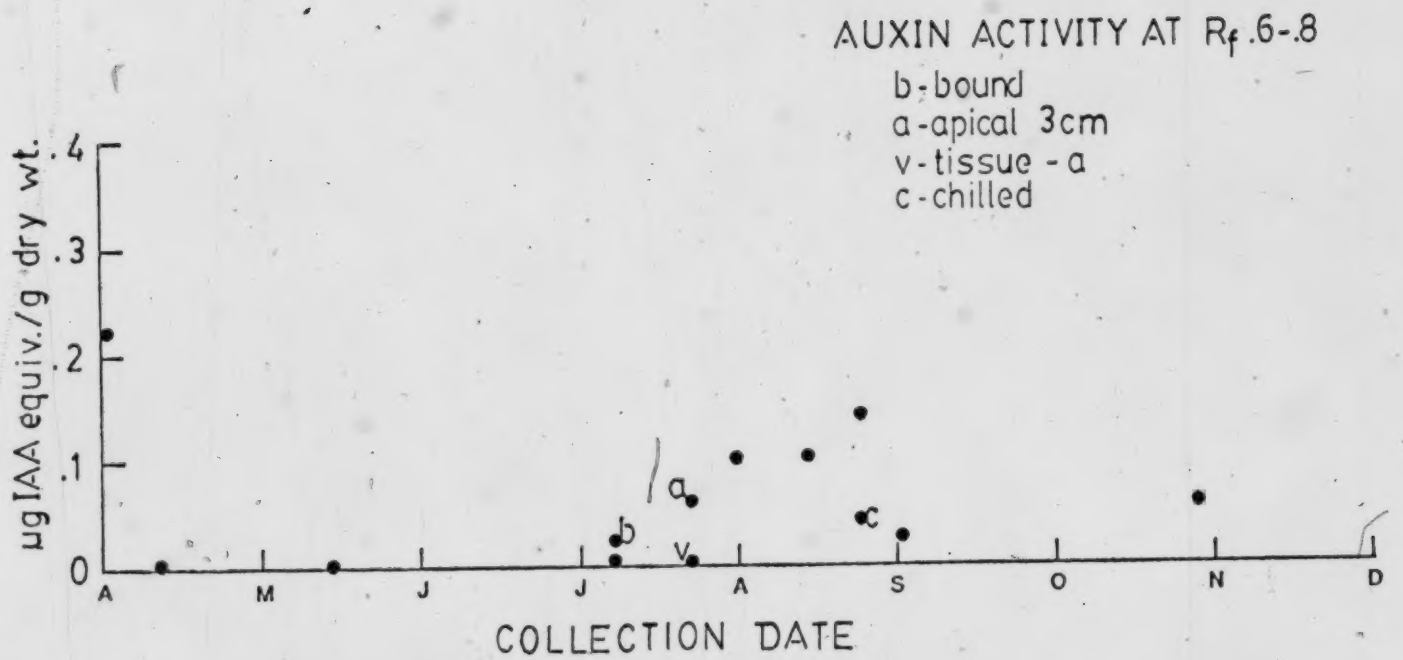

Fig. 22. Relative levels of auxin-like activity (R_f 0.6-0.8) detected in *U. vulgaris* tissue collected during 1977. Samples collected or treated differently are indicated by the appropriate code letter (see Discussion and Conclusions).

The inhibitor at 0.2-0.4 was consistently present in turion tissue but not in other tissue. This was true even in the vegetative tissue sampled immediately below the forming turion. Relative levels of this inhibitor over the season can be seen in Fig. 23. The inhibitor was found in the highest concentrations from July 28 to September 1 and completely inhibited every bioassay. In view of its association with turion tissue this inhibitor was bioassayed with turions which were ready to sprout (collections after October 2) to check for sprouting inhibition. As can be seen in Plate 3 this compound(s) stimulated sprouting. It was observed that this inhibitor acted as an antibiotic in the culture and prevented obvious algal and bacterial growth.

The inhibitor at R_f 0.5-0.7 was detected fairly consistently over the sampling period (Fig. 23). In late March, mid-April and mid-May there were no detectable levels of this inhibitor in the free form. The mid-April tissue had low levels of the bound component while the mid-May sample had a high level of the bound form. Early July showed no bound inhibitor activity while the level of the free inhibitor was high. Analysis of extracts from collections over the period from early August to late October showed a rather steady decline in the levels of the free inhibitor while the bound component was not detectable. Artificial chilling of turions collected on August 23 for 40 days caused a significant increase in the levels of this inhibitor in the free form.

Levels of Gibberellins

There was gibberellin-like activity detected at three R_f

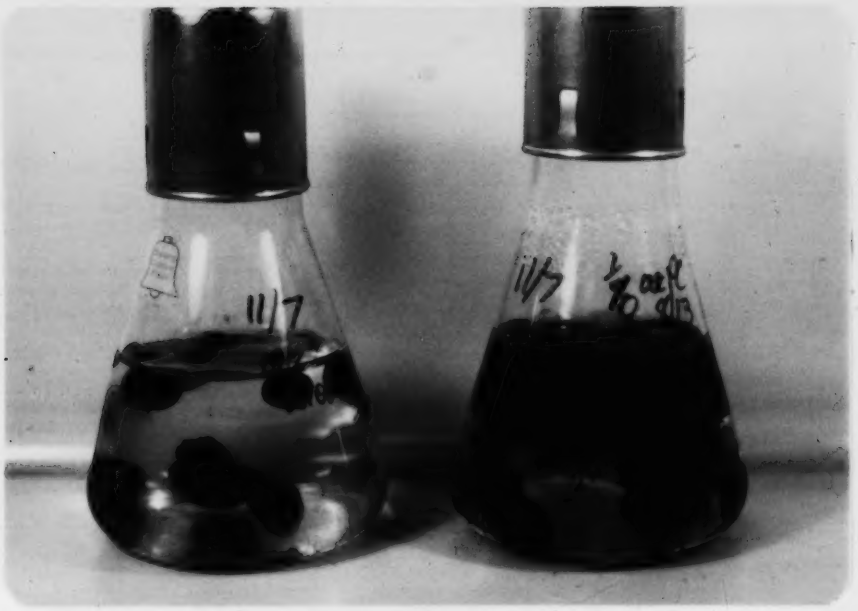

Plate 3. The effects of an endogenous inhibitor (R_f 0.2-0.4) (on right) on the sprouting response of *U. vulgaris* turions collected on August 13, 1977 and tested on November 7, 1977.

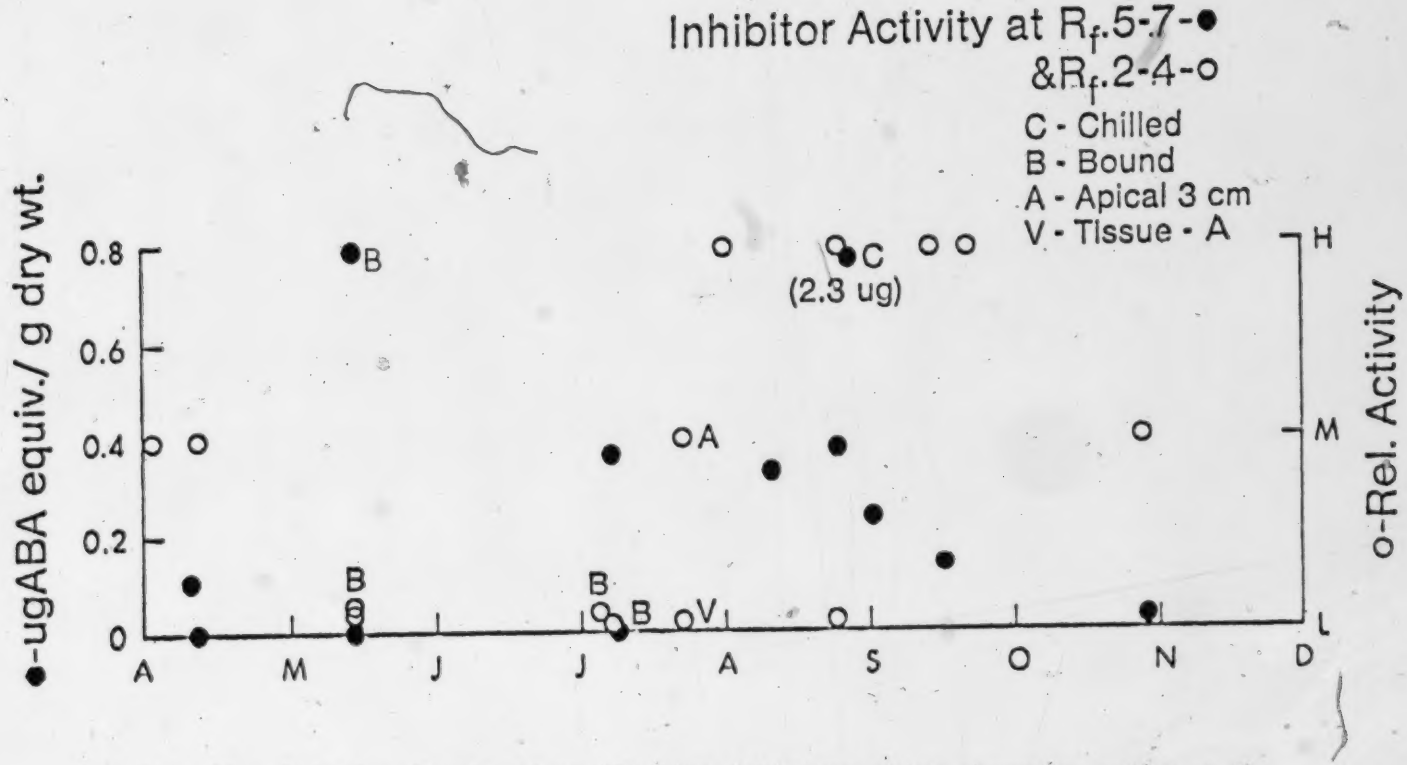

Fig. 23. Relative levels of an ABA-like inhibitor which chromatographed at R_f 0.5-0.7 and of another unknown inhibitor (R_f 0.2-0.4) detected in extracts of *U. vulgaris* tissue collected during 1977. Samples collected or treated differently are indicated by the appropriate code letter (see Discussion and Conclusions).

zones. The most consistent activity appeared at the origin (R_f 0-0.2) with less consistent activity occurring at R_f 0.4-0.5 and R_f 0.8-0.9.

Activity at R_f 0-0.2 (Fig. 24) was high in early April but fell to a barely detectable level in mid-May. The mid-May tissue had significant levels of the bound component of this gibberellin-like substance. Tissue collected during the first week of July had very high levels of the bound form but low levels of the free gibberellin. During this period the turion-forming tissue (apical 3 cm) had higher levels of bound gibberellin-like activity than did the lower portion of the shoot. GA-like activity in turions collected at the end of July was high and increased further in a sample collected at the end of August. Artificial chilling for 40 days caused a drop in the level of the free component. A late October sample had a level of activity of the free form greater than the level found in the previous sample. The bound component was not detected at this time.

Detection of gibberellin-like activity at the other two R_f zones was very sporadic (Fig. 25). Gibberellin-like activity at R_f 0.8-0.9 was low in mid-April but high in mid-May. This activity was detected only once again; in very low levels in the tissue collected in late September. Activity at R_f 0.4-0.5 was detected in only 3 samples. It was present in a free form in low levels in mid-May and late October. The bound form of this gibberellin-like substance was present in high amounts in early July. Again, the bioassay inhibitor at R_f 0.2-0.4 could have interfered with detection.

Levels of Cytokinins

Although recovery of cytokinins (with kinetin used as the model compound) was very poor, cytokinin activity was occasionally

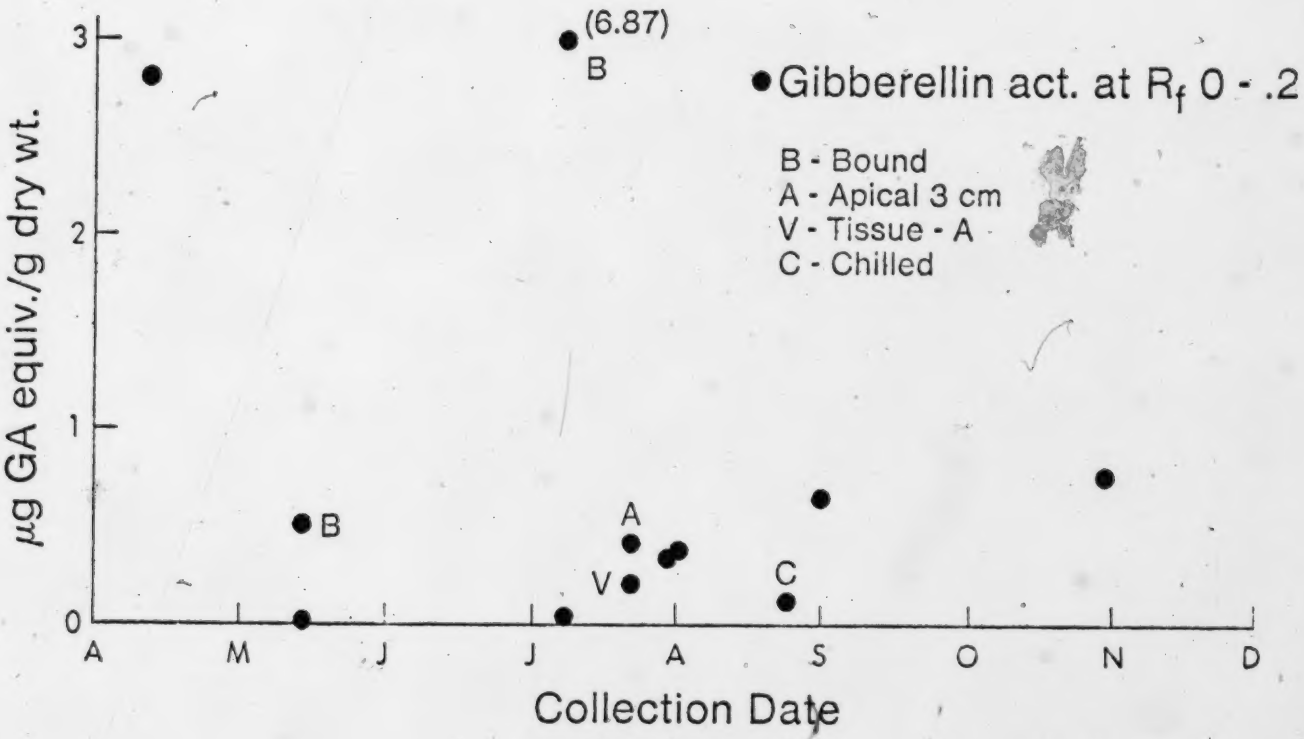

Fig. 24. Relative levels of gibberellin-like activity (R_f 0-0.2) detected in extracts of *U. vulgaris* tissue during 1977. Samples collected or treated differently are indicated by the appropriate code letter (see Discussion and Conclusions).

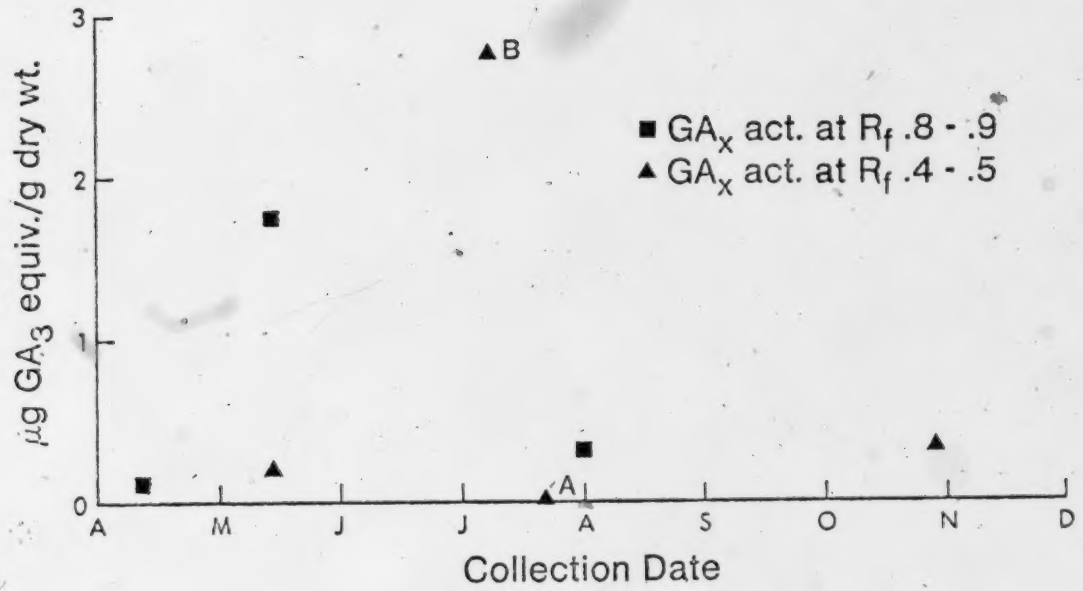

Fig. 25. Relative levels of two gibberellin-like activities (R_f 0.8-0.9 and 0.4-0.5) detected in extracts of *U. vulgaris* tissue during 1977. B indicates extraction of bound components while A indicates sampling of apical 3 cm of shoot tissue only.

detected at three R_f zones; origin, R_f 0.1-0.3 and R_f 0.6-0.7 (Fig. 26).

At the origin and at R_f 0.6-0.7 activity was fairly high in early April. No cytokinin activity was detected in extracts from tissue collected from that point up to the third week of July when activity at R_f 0.6-0.7 was detected. A late July sample had measurable levels of the R_f 0.6-0.7 cytokinin-like activity in addition to some activity at the origin. Tissue collected in late August had activity at R_f 0.6-0.7 and R_f 0.1-0.3. Artificial chilling of these turions for 40 days caused a disappearance of R_f 0.6-0.7 activity and appearance of activity at the origin. Mid-September turions had low levels of activity at R_f 0.1-0.3 and at R_f 0.6-0.7. Turions collected in late October had no detectable levels of cytokinins.

Effects of Exogenously Applied Growth Regulators on Dormant Turions

As turions were being formed and becoming increasingly dormant it was of interest to see if the inhibiting, endogenous growth regulator balance could be compensated for by exogenous application of growth-promoting substances.

Upon first observation of turion formation a collection was made and the response of these turions to various concentrations of kinetin and GA_3 was investigated. The results of this experiment can be seen in Fig. 27. The two highest concentrations of kinetin (1×10^{-4} M and 1×10^{-5} M) elicited the most complete and immediate response (100% and 50%, respectively). Forty per cent of the turions exposed to 1×10^{-4} M GA_3 sprouted while lower concentrations of GA_3 and kinetin and the control sprouted in similar percentages (0-30%).

A replicate experiment done with more completely dormant turions gave less ambiguous results (Fig. 28). The highest concentra-

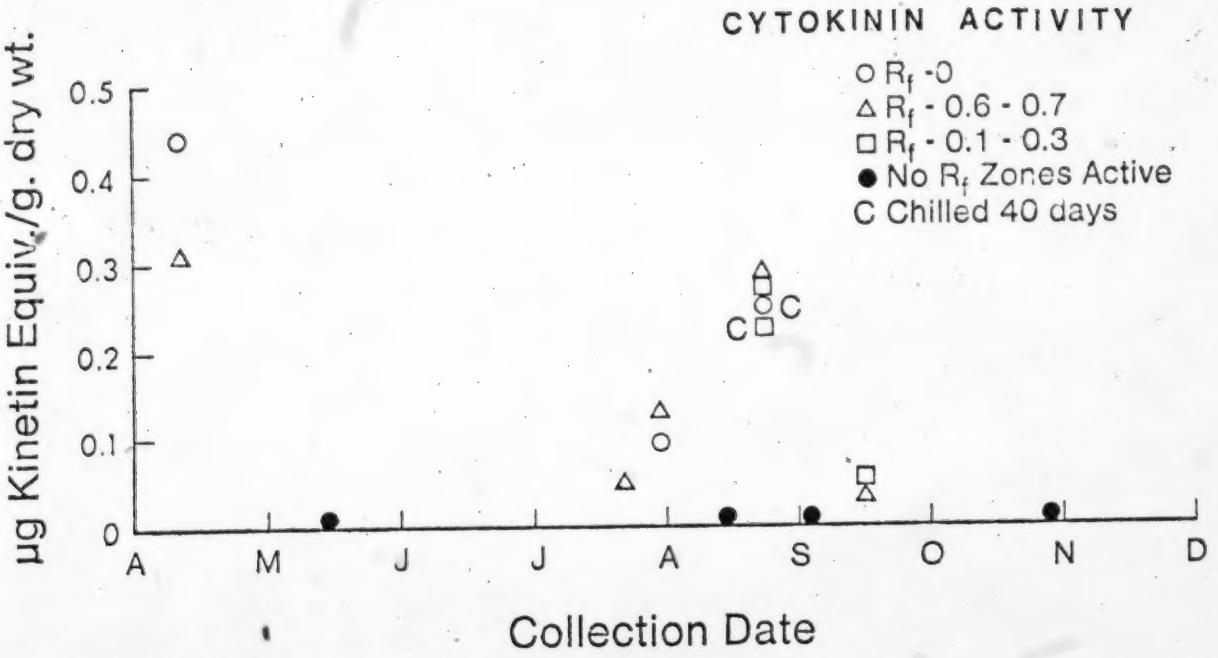

Fig. 26. Relative levels of cytokinin-like activities (R_f 0, 0.1-0.3 and 0.6-0.7) detected in extracts of *U. vulgaris* tissue during 1977. Samples collected or treated differently are indicated by the appropriate code letter (see Discussion and Conclusions).

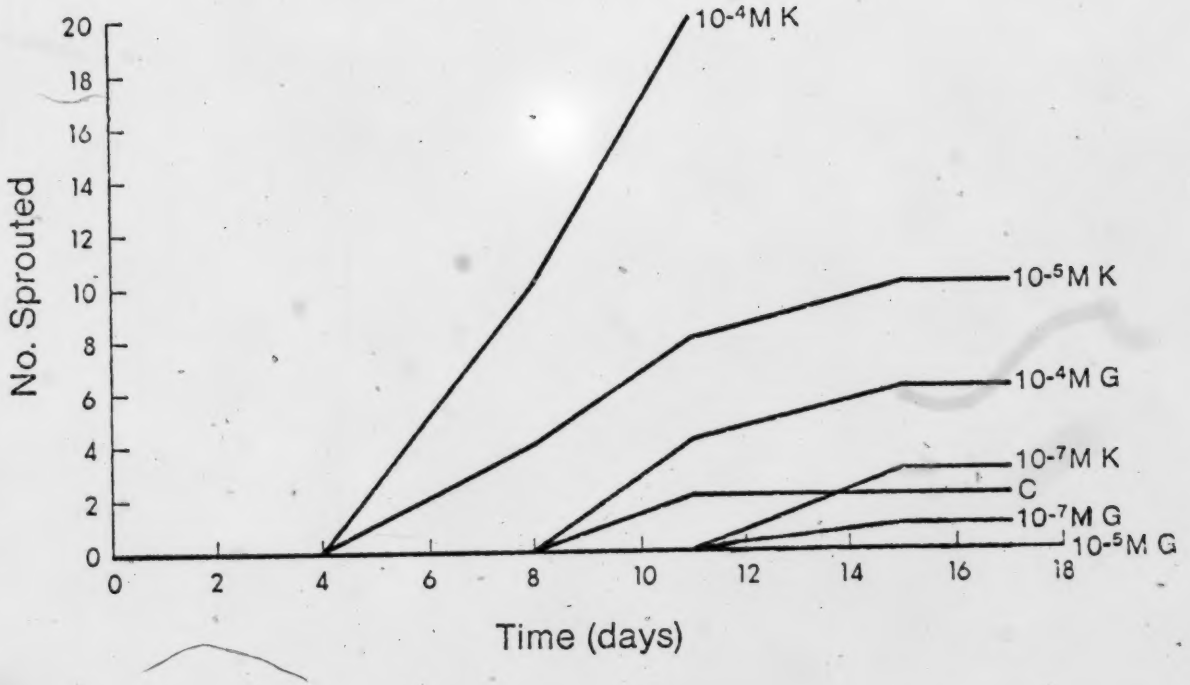

Fig. 27. Sprouting response of lots of 20 turions collected on July 21, 1977 to various concentrations of kinetin (K) and gibberellic acid (G).

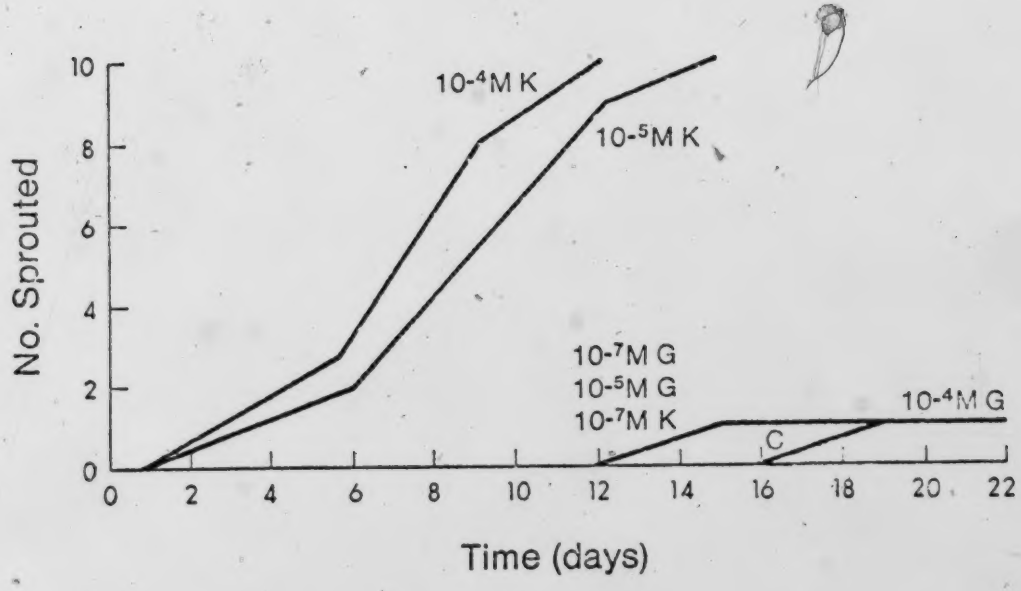

Fig. 28. Sprouting response of lots of 10 turions collected August 6, 1977 to various concentrations of kinetin (K) and gibberellic acid (G).

tions of kinetin (1×10^{-4} M and 1×10^{-5} M) caused immediate sprouting while the lower concentration of kinetin, all GA_3 concentrations and the control did not sprout.

Two additional replicates, one of which included concentrations of IAA (Fig. 29), gave identical results and confirmed that kinetin alone in concentrations equal to or greater than 1×10^{-5} M caused sprouting in dormant turions.

Dormancy Maintenance by Use of Exogenous Growth Regulator Application

Turions which would sprout (chilled adequately or subjected to HT treatment) were used to explore the possibility than an externally supplied growth substance could maintain dormancy.

Two replicate flasks containing 1×10^{-5} M ABA solution and two containing distilled water were placed in SD, LD and high temperature conditions with 10 turions each. Those placed in HT conditions and containing distilled water sprouted within 3 days while the ABA solution maintained dormancy for an undetermined period of time. Replacement of the ABA solutions with water proved turion viability. Identical results were obtained in the SD and LD chambers with the exception that longer periods with required for sprouting of the controls.

The results of an experiment done with various concentrations of ABA can be seen in Fig. 30. This experiment was conducted in the HT chamber to enhance sprouting speed. Even the lowest concentrations of ABA (1×10^{-11} M and 1×10^{-10} M) caused some inhibition. The highest concentration (1×10^{-5} M) completely inhibited sprouting.

The possibility that IAA could inhibit sprouting was tested with 1×10^{-4} M and 1×10^{-6} M solutions but sprouting rate did not

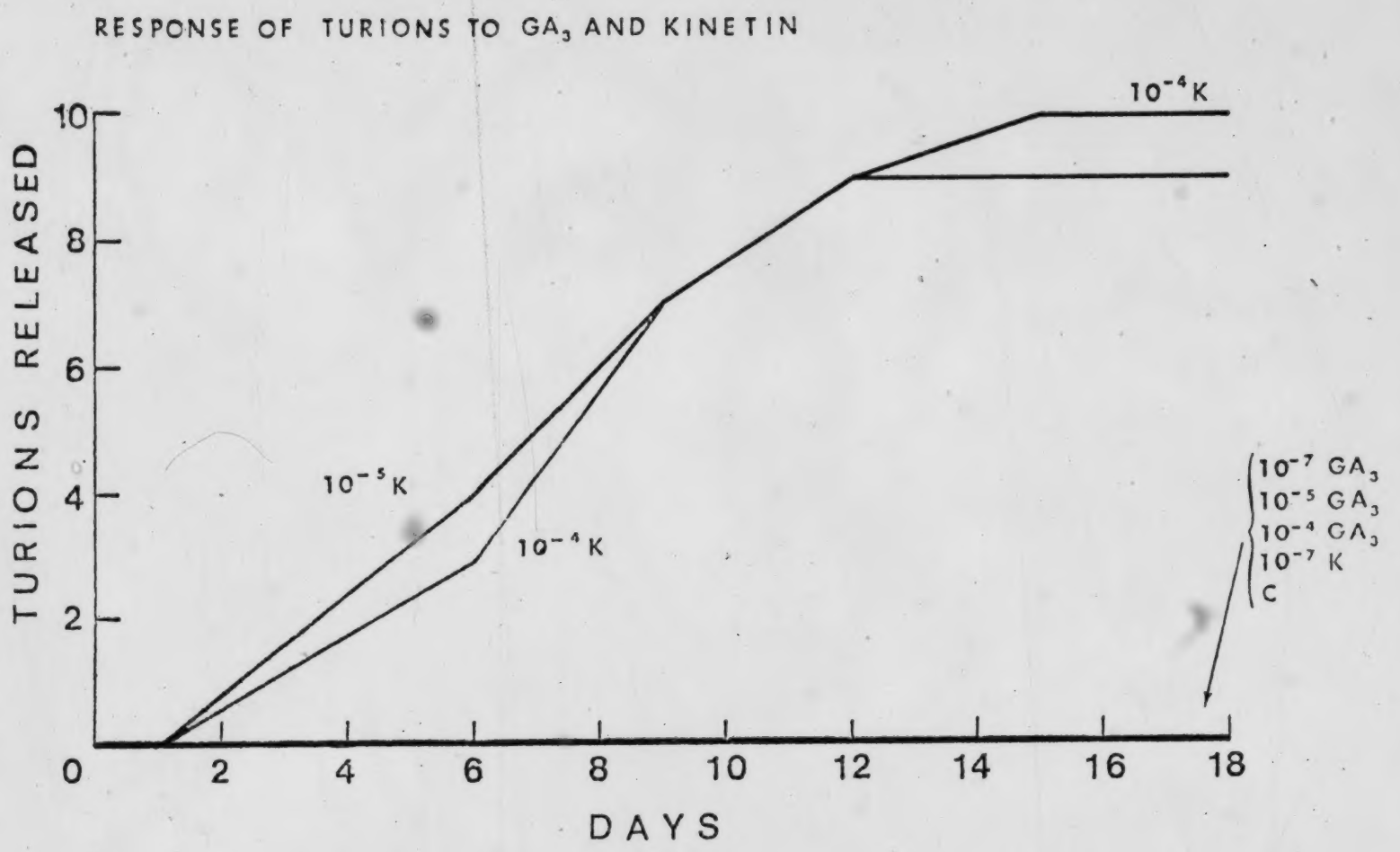

Fig. 29. Sprouting response of lots of 10 turions collected Aug. 6, 1977 and chilled for 3 weeks to various molar concentrations of kinetin (K) and gibberellic acid (GA_3).

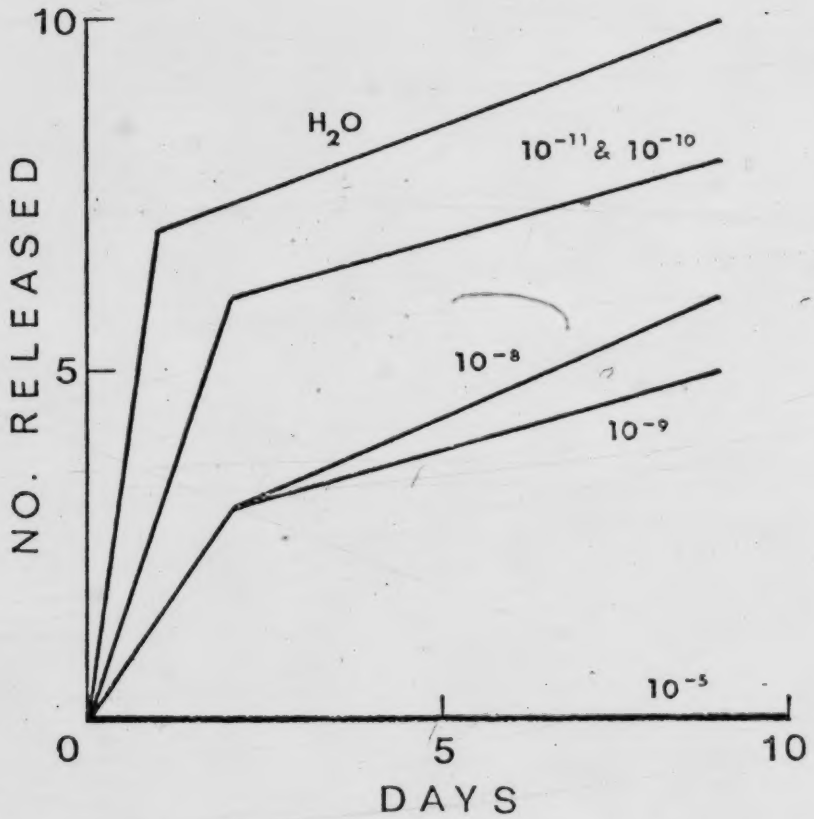

Fig. 30. Sprouting response of lots of 10 turions collected on Oct. 27, 1977 to various molar concentrations of ABA.

differ from controls.

A Preliminary Experiment Investigating Prevention of Secondary Dormancy

As can be seen in Table 10 turion reformation occurred consistently in tissue collected on or prior to August 23 regardless of photoperiod at 20 C. It was of interest to see if exogenous growth substance applications could prevent this and allow for continued growth. Only one preliminary experiment was conducted, the results of which can be seen in Table 10. In this experiment only a combination of GA_3 and IAA prevented turion reformation while IAA alone seemed to inhibit turion reformation and GA_3 and kinetin had no effect.

Turion Induction by ABA

The results of ABA application to actively growing tissue were quite clear in that turions were consistently produced within one week when correct concentrations were used. Tissue collected on March 18 and grown in LD conditions for four months produced turions within one week when placed in a 1×10^{-5} M ABA solution. Vegetative tissue collected on May 28, 1978 and grown in LD conditions for one month produced turions within one week when placed in a 1×10^{-5} M ABA solution. Both the above experiments had distilled water controls which did not produce turions. Another experiment showed that 1×10^{-6} M ABA concentration would also induce turion formation in all apices used. This was confirmed by another experiment in which all apices (3) formed turions when incubated with 1×10^{-6} M ABA solution when incubated in LD, non-inductive conditions.

Table 10. Average of response of lots of 12 plants obtained from heat-treated turions (collected August 2, 1977) to various growth substances in terms of turion reformation.

TURIONS COLL. AUG. 2 , n=12

TREATMENT	NO. TURIONS REFORMED
C	7
10^{-5} M GA	7
10^{-6}M IAA	2
10^{-5}M GA + 10^{-6}M IAA	0
10^{-5}M KINETIN	6

Starch Content

The change in starch content over the season can be seen in Fig. 31. Starch content is low in the turion in early April. In mid-May starch level reaches a minimum of 0.638 mg · g^{-1} dry weight. As turion formation is in progress in the third week of July, starch accumulation begins and accelerates to reach a maximum in the second week of August of 1.49 mg · g^{-1} dry weight. From this date levels begin to decline over the dormant season to the last sample date of October 27 which has 0.817 mg · g^{-1} dry weight, just slightly above the level found in the previous spring. Artificial chilling of turions collected on August 23 for 40 days caused a significant drop in starch content which was roughly equivalent to the level attained if natural chilling had occurred for the same period.

Dark Respiration Measurements

Dark respiration in response to temperature was measured for two sets of tissue in two separate experiments. Tissue was obtained from turions which had a potential to enter secondary dormancy (collected September 2) and from turions which, once sprouted, would not reform turions (collected October 27). The results are shown in Fig. 32. The tissue from turions collected on September 2 exhibited a break in the linear response of respiration to increasing temperature of 25 C ($33.6 \times 10^4/T°K$). The tissue from turions collected on October 27 exhibited a linear response of respiration to increasing temperature above 25 C.

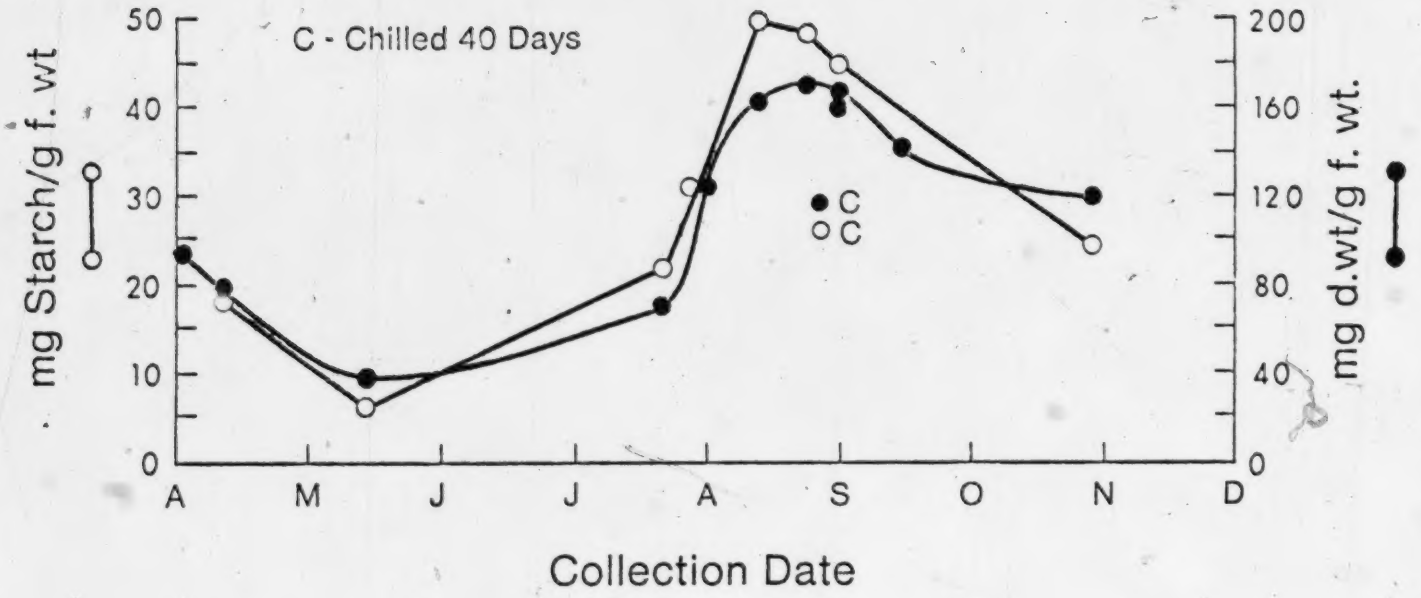

Fig. 31. Change in dry weight and starch content of *U. vulgaris* plants during 1977.

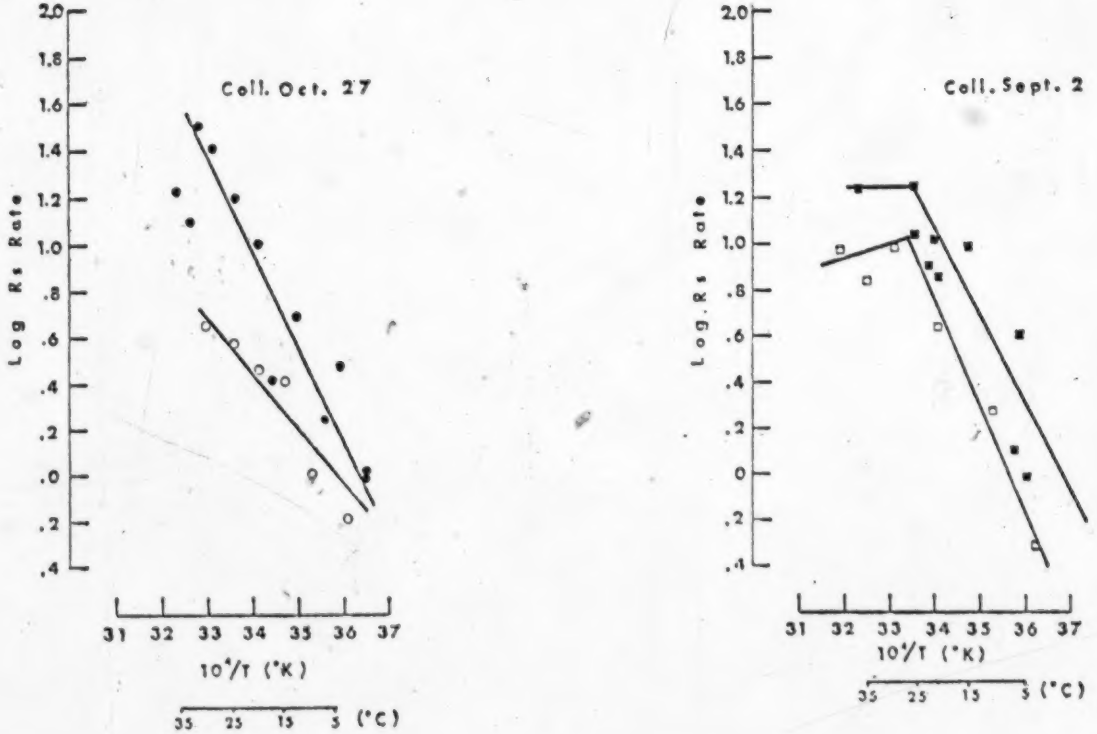

Fig. 32. Arrhenius plots of respiration of two sets of *U. vulgaris* tissue obtained from turions which had been sprouted for 2 (□ ○) and 3 (■ ●) weeks prior to each experiment. The sprouted plants had been grown under LD conditions.

DISCUSSION AND CONCLUSIONS

Utricularia vulgaris enters a period of dormancy by the production of a specialized structure, the turion. Dormancy is induced primarily in response to periodic environmental conditions which precede winter but can also be induced by other stress-associated factors. During these stress periods the life cycle of *U. vulgaris* is carried on solely by the turion and as such it exhibits significant changes in growth potential and basic physiological states. The fundamental controlling agent in dormancy of terrestrial as well as aquatic plants is the physical environment. Environmental change is perceived by largely unknown mechanisms which result in altered balances of growth regulating substances and these, in turn, are manifested by developmental and physiological changes. The precise mechanism of this information translation is unknown but correlations between changes in environmental factors, endogenous concentrations of growth regulators and plant responses for many species supports the hypothesis that these are functionally related.

The turion of *U. vulgaris* is a terminal bud formed at the apices of viable main shoots and branches. It is composed of specialized scale-like leaves which are tough, hairy and less divided than typical vegetative leaves. The turion of *U. vulgaris* is very sturdy and does not rely, as does the actively growing plant, on the water medium for support. The *Utricularia* turion is homologous to the overwintering bud of woody plants in terms of structure. It has specialized outer leaves for protection in the same way that buds have cataphylls or stipules. The telescoped shoot(s) with many of next season's leaves present is also similar to the structure of woody

plant buds (Romberger 1963). In addition to protective features, the turion has numerous, well-developed gland-like trichomes which produce a mucilage matrix and covering. Many submerged aquatic angiosperms produce specialized shoot tips for overwintering and stress survival (Sculthorpe 1967) but few have attained the degree of specialization exhibited by the turion of *U. vulgaris*. The formation of sclerophyllous leaves with many hairs, copious mucilage, and an overall sturdy construction are modifications that ensure survival during extreme environmental conditions not encountered by most submerged aquatic angiosperms (which are true cryptophytes).

The turion of *U. vulgaris* serves several purposes. The primary function is that of an overwintering structure (Sculthorpe 1967 and Arber 1920) although it is also produced during other periods of stress such as desiccation (Maier 1973b) and nutrient deficiency (H. Gluck and P. von Leutzelburg in Sculthorpe 1967). The turions of *U. vulgaris* form well in advance of deleterious environmental conditions and remain buoyant throughout the winter. The buoyancy results in exposure to extremes of environmental stress, since,during freeze-up, some may become embedded in ice at the air-ice interface with part of the turion exposed to air (Plate 2). It was found that turions taken from the ice had developed a certain degree of frost hardiness. They could survive temperatures as low as -8C but not -12C. Early observations support the idea that *U. vulgaris* turions are particularly well-suited to surviving if embedded in ice compared to the turions of other species (H. Gluck in Arber 1920). According to Mazur (1969) the cell surface blocks ice crystal seeding in the cytoplasm at temperatures above -10C. As no turions were found to be solidly frozen during the study period it is likely that snow cover and ice considerably mitigate

the effects of very low air temperatures which occurred such that supercooling or an osmotically lowered freezing point provided sufficient protection from freezing. The formation of turions in aged or nutrient-depleted cultures was also observed in this study. The influence of nutrient availability on turion formation was supported by transfer of these cultures to fresh media and the observation that this resulted in turion outgrowth. From the findings reported in this study plus those of Maier (1973b), it can be seen that dormancy is a response to varied, threatening environmental conditions which enables the population to survive periods of stress. The turion of *U. vulgaris* is homologous with the bud of woody plants in terms of function as well as structure.

A secondary function of the *U. vulgaris* turion is the propagation of the species. It was the only means of propagation observed during this study although viable seeds were produced. A possible reason for the failure to observe seedlings is that plants from both seed and turion had to compete for the same niche. The plants from turions were rapid growers and could, after 2 months, reach lengths up to 1.0 m with many shorter side shoots whereas seedlings, grown in the laboratory for a similar period, under conditions favorable for growth from sprouted turions, achieved no more than 10 cm of growth. The buoyant turion was also well-suited for dispersal as, after overwintering, it would become detached from the degenerated parent and free to float about. This characteristic is important for dispersal because, even though *U. vulgaris* is described as a free-floating plant, during the growth season the basal portion of the shoot becomes senescent and covered with detritus which causes attachment to the bottom and prohibits movement. In addition, the large size of the

plant inhibits movement. The seed is submerged and thus at a disadvantage with respect to dispersal. The dispersal function has been described for turions of *Myriophyllum exalbescens* (Weber 1972). *M. exalbescens* was found to be primarily, if not exclusively, propagated by turions in studies involving a lake in Michigan. Other aquatic angiosperms such as *Elodea canadensis*, also depend solely on vegetative propagation (Sculthorpe 1967).

At the study site, *U. vulgaris* was observed to initiate the formation of turions during a period of high temperature and decreasing daylength when flowering was nearly completed and most plants had developing fruit. Every plant examined had developing turions. These observations suggested two possibilities: 1) turions were induced by decreasing daylength, or 2) turions were induced by an endogenous developmental rhythm. While the latter possibility is interesting, especially in view of a recent report of a senescence signal originating in developing fruit of soybean (Lindoo and Nooden 1978), it was not investigated during this project. The occurrence of turions on fruitless plants tends to discount this possibility, however. The first possibility was tested since it is a hypothesis which is supported by experimental evidence for many species.

Laboratory experiments with *U. vulgaris* showed that short days induced turions. This response was enhanced by concomitant low temperature. While the summer solstice had occurred only one month prior to the first observation of turions it has been shown that turion induction in *Myriophyllum verticillatum* by short days is much enhanced by a long day pre-treatment (Weber 1976b). Thus it is possible that the plant may be sensitive to a reversal of the changing

daylength from increasing to decreasing periods. Turions of other aquatic angiosperms, such as *Hydrocharis morsus-ranae* (Terras 1900), are also sensitive to daylength. In the hemicryptophyte *Pinguicula grandiflora* (Lentibulariaceae) short days induce the formation of bud-like hibernaculae (Heslop-Harrison 1962). Induction of bud dormancy in woody plants has long been known to be a response to short days and/or cool temperatures (Wareing 1956). So it appears that in *U. vulgaris* daylength, or changing daylengths, serve as the environmental cue to initiate turion formation as is the case for many other plant species that undergo morphological changes in preparation for winter.

As turions formed and developed they were tested for sprouting potential. These tests resulted in a recognizable pattern of dormancy. The stages of dormancy; pre-dormancy, innate dormancy and imposed or post-dormancy, as described by Wareing (1969a), Vegis (1964) and others for woody plant buds were also present in *U. vulgaris* turions. Pre-dormancy, during which the turion is held dormant by factors arising outside the organ itself, was very short in *U. vulgaris*. In fact only the collection on the date which turions were first observed showed significant sprouting when detached from the parent shoot. Subsequent collections were increasingly more difficult to sprout and were dormant due to internal factors. These were in the stage of innate dormancy. During this period of innate dormancy the parent plant was senescencing and was likely losing the ability to regulate dormancy in the turion. Environmental conditions were favorable for growth at this time. Collected turions remained dormant for an undetermined period of time under controlled environment at 20C (+ 4 months) but with various degrees of delay, sprouted at 30C. The time

required to obtain sprouting at 30C was a good measure of the depth of innately controlled dormancy. Dormancy release at 30C is similar to the release of dormant buds of woody plants by a warm water treatment as described by Samish (1954). Release of dormancy and sprouting in these experiments was defined by leaf reflexing and stem elongation. Plants obtained from innately dormant turions would continue to grow vegetatively at 30C but would reform turions and become dormant if grown at 20C, even in long days. While the high temperature release of dormant *U. vulgaris* is mentioned by Vegis (1964) and Maier (1973a) the reinstatement of dormancy has not been previously described. This phenomenon is analogous to the recurrence of correlative inhibition of lateral buds forced to sprout by a kinetin treatment (Phillips 1969). The use of heat to release correlative inhibition of lateral buds has not been reported.

Turions exhibited an increasing readiness to sprout at 20C and a more immediate response at 30C, which was roughly correlated with deteriorating environmental conditions. At this time the turions were entering the third and final stage; imposed dormancy (or post-dormancy) during which dormancy was maintained by environmental factors unfavorable for growth. Once they were fully in the imposed dormancy stage the recurrence of dormancy at 20C following a short period of growth was not observed. Plants from turions collected at a transition point from innate to imposed dormancy appeared to be sensitive to photoperiod and responded to short days by reforming turions and to long day by continuing growth. Turions which sprouted during the imposed dormancy stage formed much larger and more robust plants with less mucilage than those obtained from plants collected earlier. Also plants from imposed dormancy turions produced structures,

airshoots, heretofore unseen with sprouted turions. Another aquatic angiosperm, *Ceratophyllum demersum*, has also been reported to have periods of innate and imposed dormancy (Best and Soekarjo 1976). Turions of *Myriophyllum verticillatum* show a sprouting response to favorable environmental conditions up to a point after which a cold treatment is necessary to obtain sprouting (Weber 1976a).

While the turion undergoes no obvious morphological change during dormancy it does experience large alterations in physiological state as demonstrated by the change from innate to imposed dormancy. An indication of metabolism is the fluctuation of starch level. Starch is a primary storage carbohydrate in *U. vulgaris* turions. Changes in starch level account for up to 50% of the dry weight changes observed during dormancy. Starch levels reached a maximum at the end of the innate dormancy period and fell sharply as environmental conditions deteriorated and imposed dormancy occurred. In *Myriophyllum verticillatum* starch is the major storage carbohydrate but changes in level did not account for all of the total dry weight changes during growth and development (Weber 1973). Romberger (1963) cites an example of seasonal change in carbohydrate reserves in a woody plant, *Fagus sylvatica*. The major component was at a maximum in early autumn and began to increase again in mid-winter. In the twigs of most woody plants it has been found that total carbohydrate has an autumn peak and a spring minimum (Kozlowski 1971). In the inner bark of black locust this was found to occur in addition to another maximum in early summer. The potential for separate storage sites in woody plants makes comparison with *Utricularia* turions questionable, however the pattern of change is similar with an autumn (or early dormancy) peak and a spring minimum.

An attempt was made to differentiate between innate dormancy and imposed dormancy stages on the basis of respiration rate of sprouted turions in response to increasing temperature. Respiration response was expressed in Arrhenius plots. Tissue from turions collected during the innate dormancy stage and sprouted exhibited a break in respiration response at high temperatures while that from turions collected during the imposed dormancy stage did not. This difference in tissue response to increasing temperatures is of unknown origin but it can be seen that this feature enables the dormant plant, which must reform a turion due to internal factors, to conserve its carbohydrate reserves. The plant which has the potential to continue growth and development does not alter its respiratory response at high temperatures. A shift, rather than a break, in Arrhenius plots of respiration *vs.* temperature for *Dryas integrifolia* was found to occur in dormant plants such that a 10C higher temperature was required to obtain an equivalent respiration rate to that of non-dormant tissue (Hartgerink and Mayo 1976). This characteristic also enables carbohydrate conservation by dormant plants. The experiments with *U. vulgaris* showed that in addition to other differences, tissue in innate dormancy and that in imposed dormancy had basic physiological differences.

The transition from innate dormancy to imposed dormancy takes place during a chilling period in many species [e.g. *M. verticillatum* (Weber 1976a) and *Ribes nigrum* (El-Antably in Wareing and Saunders 1971)]. Turions collected during innate dormancy and artificially chilled were compared to later collections which had been naturally chilled for the same period of time. Artificial chilling in darkness substituted for natural chilling in terms of the sprouting response but not in terms of development of a potential

for continued vegetative growth. This suggests that the two processes are under different internal control and that features of the artificial environment, such as lack of light, did not substitute for the natural environment.

That the phytochrome system was involved in photoperiod perception was suggested by the induction of turions by short daylengths. Another aspect of dormancy which proved to have a light involvement was sprouting. Turions collected during the imposed dormancy stage exhibited considerably retarded sprouting in the dark even at 30C compared to those exposed to the normal growth chamber light. A red light pre-treatment did not alleviate the inhibition but the red light source emitted large amounts of far-red light which may have reversed the red-activated phytochrome system. This experiment, therefore, does not eliminate the possibility that the phytochrome system is involved in dormancy control of *U. vulgaris*. The sprouting of turions of *Spirodela polyrhiza* requires light and is a red, far-red reversible phenomenon (Czopek 1964) and dormancy of many woody plants is a phytochrome-mediated response (see Vince-Prue 1975). Light was not found to be required for sprouting of *Myriophyllum verticillatum* turions, however (Weber 1973).

To sum up, therefore, the chronology of dormancy in *U. vulgaris* is similar in many respects to that reported for other turion-forming aquatic angiosperms and to the basic patterns found in bud-forming woody plants. In the natural situation turions are formed one month following the summer solstice. Under controlled conditions, short days with or without associated cool temperatures, induce turions. For a short period after being formed the turions will sprout if detached and placed in favorable growth conditions.

The parent plant is therefore implicated in the induction and maintenance of dormancy at this time. This is pre-dormancy and in many woody plants buds will sprout if the leaves of the tree are removed, thereby indicating the source of the inductive stimulus. Following pre-dormancy, turions of *U. vulgaris* reach a stage in which sprouting is most difficult to obtain. This is the period of dormancy controlled by innate factors. If the turion is forced to sprout (by heat or growth regulator treatment) at this stage the resulting shoot will elongate only for a limited period at which time a turion will reform and dormancy will ensue. This response suggests that sprouting and growth are internally controlled by separate factors and also that sprouting is not necessarily an accurate indicator of dormancy release. The early period of dormancy is accompanied by rapid starch accumulation in the turion. Thus, turions were induced and maintained dormant by internal factors at a time when the favorable environmental conditions permitted food storage by the parent plant. It was also found that the morphological features of the turion allow the structure to survive a degree of desiccation, mechanical stress and sub-freezing temperatures. Numerous aerenchyma cells allow the structure to be continually floating thereby increasing environmental exposure and facilitating dispersal.

By mid fall the turion has entered the stage of imposed dormancy. This transition may have been induced by deteriorating environmental conditions, by internal physiological developments or a combination of both of these factors. Dormancy is not maintained by internal factors and the turion will sprout and continue to grow if collected at this time. Turions collected prior to this time and stored just above freezing develop the potential to sprout like their naturally chilled counterparts but would re-enter dormancy by producing a turion after a period of growth. One factor not

provided the artificially chilled turions was the considerable light received by those in the field. Of course many other factors were different for the two groups, any one of which could have been critical. This observation does, however, indicate a separation in the control of sprouting and subsequent growth. The response of respiration to increasing temperatures for tissue from sprouted turions collected during both innate and imposed dormancy also showed a difference between the two groups. The tissue which was committed by internal factors to re-enter dormancy showed a failure of the respiratory system in response to extremely high (but not unnatural) temperatures. Tissue collected during imposed dormancy continued to respond normally to the highest temperatures tested. Thus, the turion of *U. vulgaris* undergoes two primary stages of dormancy; innate and imposed. The first allows dormancy to occur during a period of favorable growth conditions and allows for the accumulation of stored carbohydrates. The internal control is a fail-safe "mechanism" whereby sprouting can be induced by abnormal circumstances but growth and use of food reserves is otherwise severely restricted. These controls are probably on two levels; balances of endogenous growth substance and basic metabolic (biochemical) limitations. The second stage occurs as the environment deteriorates and internal control is lost. At this time dormancy is maintained by external factors, the removal of which results in rapid sprouting and growth.

The following shows how various environmental factors relate to the stages of dormancy in *U. vulgaris*:

1. Short Day
   SD + low temp
   SD preceded by LD = Dormancy induction
   Nutrient depleted medium
   Desiccation

2. Low temp + unknown = Removal of innate dormancy

3. Low temp
   Low light levels = Dormancy maintenance

4. Warm temp + Long Day = Sprouting and growth

The other aspect of dormancy in *U. vulgaris* investigated in this study was the involvement of growth regulators. The problem of growth regulator control was approached in two ways: by determination of endogenous levels and by observing the response of various tissue to exogenous application of synthetic growth regulators. In this fashion evidence obtained by one approach could be compared to that from the other. The four major classes of growth regulators investigated were auxins, inhibitors, gibberellins and cytokinins. Endogenous levels of growth regulators were measured by extraction, chromatographic separation and bioassay of the chromatographic sections, expressing the results as synthetic growth regulator equivalents. As rigorous identification of the growth substances was not made the endogenous substances were defined by the particular bioassay in which they were active and identified by the R_f value at which they were found in a solvent system composed of 2-propanol:ammonia:water (PAW,10:1:1). Thus for auxin-like compounds two zones of activity were found; R_f 0.4 and R_f 0.6-0.8. Two inhibitor zones were located at R_f 0.2-0.4 and R_f 0.5-0.7. The inhibitor chromatographing at R_f 0.2-0.4 was always present to some extent in the turion. This inhibitor was not found to either induce turion formation or inhibit turion sprouting in preliminary experiments (Plate 3). While the nature of this inhibitor which affected all bioassays used was interesting and perhaps important in fully understanding the dormancy phenomenon in *U. vulgaris*, insufficient time prevented detailed investigation into its physiological properties

and chemical nature. Gibberellin-like compounds were located at R_f 0-0.2, R_f 0.4-0.5 and R_f 0.8-0.9. Cytokinin-like activity was found at the origin, R_f 0.1-0.3 and R_f 0.6-0.7. Due to limited extraction efficiency of a model cytokinin and the dubious value of the resulting bioassay results, data from these experiments will be discussed only briefly. Some plant extracts were also subjected to alkaline hydrolysis in order to release bound (probably sugar ester) forms of the acid growth regulators (ABA, IAA and gibberellins). Growth regulator involvement in dormancy of *U. vulgaris* was studied in relation to 3 main stages; dormancy induction and pre-dormancy, innate and imposed dormancy maintenance and dormancy release and growth. The sample collected at the initiation of turion formation was divided into an apical portion including only the developing turion and a vegetative portion consisting of the apical 3 cm of shoot without the turion. The final special sample was collected during innate dormancy, was artificially chilled for 40 days and then extracted.

Just prior to the first observation of turion formation in the field the endogenous growth regulator balance was as follows: low auxin-like activity, maximum free inhibitor activity at R_f 0.5-0.7 (ABA-like), low free gibberellin (GA_n)-like activity and high bound GA_n-like activity at R_f 0-0.2 and R_f 0.4-0.5.

Low endogenous auxin levels have not been correlated in a casual way with bud dormancy but high levels have, notably with inhibition of lateral buds in apically dominant plants. Inhibitors which are similar to the one(s) found in this study in terms of activity and R_f localization (R_f 0.5-0.7) have been found to correlate

well with changes in growth status. This inhibitor is termed inhibitor β (Bennett-Clark and Kefford 1953) and contains as the primary active component abscisic acid (ABA). In developing turions of *Myriophyllum verticillatum* the levels of this inhibitor were high relative to those found in non-dormant tissue (Weber 1976b). Dormancy in woody plants has been studied much more extensively with regard to this inhibitor. *Acer pseudoplatanus* (Phillips and Wareing 1958) and *Betula pubescens* (Kawase 1961) were found to have increased levels of this inhibitor when the plant was exposed to short day, dormancy-inductive photoperiods. *Salix viminalis* entering dormancy was found to have increasing levels of this inhibitor (Bowen and Hoad 1968). Dormant buds of *Ribes nigrum* and *Fagus sylvatica* were found to have high levels of free ABA (Wright 1975). However, as noted in the Introduction, there are a significant number of examples which do not fit into this pattern. Two species cited above, *A. pseudoplatanus* and *B. pubescens* were found not to have elevated ABA levels when placed in dormancy-inducing conditions although previous bioassay results implied its involvement (see Wareing and Saunders 1971). Dormancy control does not involve changing levels of ABA in all cases.

Much evidence has been gathered using exogenous applications of synthetic ABA which implicates it in dormancy control and general growth inhibition. In actively growing *U. vulgaris*, ABA induces turions within one week at concentrations of 1×10^{-5}M under non-inductive environmental conditions. In *Myriophyllum verticillatum* ABA enhanced turion production under inductive conditions but did not cause turion formation under non-inductive conditions (Weber 1976b). Turion induction by ABA has been reported for another aquatic

angiosperm, *Spirodela polyrhiza* (Perry and Byrne 1969). In woody plants the formation of dormant buds has been induced by ABA application in several species (El-Antably *et al.* 1967) and is widely accepted as a major dormancy regulator. Induction in woody plants requires continuous application for periods of up to three weeks. Both lines of evidence, exogenous application and endogenous levels, indicate a controlling role for ABA in dormancy of *U. vulgaris*.

The levels of the promotor class of regulator, gibberellins, were such that they also are implicated in dormancy control. In turions of other aquatic angiosperms, *Wolffiella floridana* (Prieterse 1971) and *M. verticillatum* (Weber 1976a and b), gibberellins were detected by bioassay methods but were in neither case associated in an apparent, causal way with dormancy. However, in other plant species, dormancy-inductive short day photoperiods and concommitant decreasing gibberellin levels have been associated with 1) cold hardiness in *Medicago sativa* (Waldman *et al.* 1975), 2) bud dormancy in *Ribes nigrum* (El-Antably in Wareing 1969b), and 3) cessation of cambial growth in *Betula pubescens* (Digby and Wareing 1966). It has been suggested (Jones 1973) that conjugation of free gibberellins is a means of rendering active gibberellins inactive. Detection of high levels of hydrolyzable gibberellin in extracts of *U. vulgaris* is supportive of this theory. GA conjugates have been found in sap of elm trees (Sembder *et al.* 1968). Such conjugation was found to be initiated by the application of synthetic ABA to barley seed tissue (Nadeau 1972), thus functionally linking the activity of these two classes of growth regulator. The inactivation of free, endogenous gibberellins has a likely function in dormancy induction.

During the period just following dormancy induction the stages of dormancy progressed from innately controlled dormancy to dormancy which was imposed by severe environmental conditions. Just as the environment underwent amelioration a period of sprouting and very rapid growth and development ensued. As innate dormancy commenced, an auxin-like component (R_f 0.6-0.8) began increasing in activity. Just prior to sprouting the levels of this auxin-like compound fell and the levels of the IAA-like compound (R_f 0.4) rose sharply. The ABA-like inhibitor was maintained at an approximately constant level except towards the end of the period when it rapidly fell. Gibberellin-like activity at R_f 0-0.2 began an increase during innate dormancy which continued up to the point of sprouting. As sprouting occurred the level of this gibberellin-like compound fell sharply and was replaced by high activity at R_f 0.4-0.5.

The auxin-like compound which showed a gradual increase during this period of dormancy was not the one which co-chromatographed with IAA and so may be an inactive form of auxin. There are reports of auxins (notably indole-3-acetonitrile) which are active in the oat coleoptile bioassay but not in other plant systems. This proposal is given support by the finding that this auxin-like compound (R_f 0.6-0.8) was not detected during sprouting while IAA-like activity was very high. Artificial chilling of a sample collected during innate dormancy lowered the activity of the R_f 0.6-0.8 auxin-like compound and increased the activity of the IAA-like compound. While this finding also supports the proposal that these auxin-like compounds are interconvertable and represent active and inactive forms, the levels of co-chromatographing inhibitors also changed with artificial chilling and could have interfered with the results.

Experiments using exogenous application of IAA were done in order to investigate two possible aspects of auxin control of dormancy maintenance and control. First, as high levels of auxin have been found responsible for the maintenance of lateral bud dormancy in apically dominant plants it was of interest to see if this was the nature of dormancy maintenance in *U. vulgaris*. It was found that high concentrations of IAA did not inhibit sprouting. The second aspect of auxin involvement investigated was in the change from innate to imposed dormancy in terms of growth potential. That the increasing level of auxin-like compound is important in discharging the dormant condition in *U. vulgaris* was indicated by preliminary experiments in which continuing the vegetative growth of plants from turions collected during innate dormancy was attempted. Evidence suggests that IAA primarily, but in conjunction with GA_3, allowed these plants to avoid the typical reentrance into dormancy. This is analogous to the situation found in kinetin-released lateral buds in which IAA and GA_3 enhance continued growth (Phillips 1975).

The levels of the ABA-like inhibitor changed in a fashion which supports the contention that it is of primary importance in the maintenance of innate dormancy. Artificial chilling of tissue appeared to increase the levels of this inhibitor. The level of this inhibitor fell just prior to the onset of imposed dormancy and remained nearly undetected throughout the sprouting and early growth period. During sprouting and active growth, a bound component of this inhibitor was detected and reached high levels. High levels of bound ABA have also been associated with bud burst in *Ribes nigrum* and *Fagus sylvatica* (Wright 1975). The levels of the ABA-like inhibitor are possibly

governed by sequestering the inhibitor in a bound, inactive form.

Using exogenously applied ABA the inferences made using endogenous data were supported. In all cases ABA inhibited sprouting, even under high temperature conditions. In *Myriophyllum verticillatum* ABA could not inhibit sprouting of cold-treated turions but could retard the sprouting of those turions not exposed to a cold treatment. ABA has been reported to inhibit the sprouting of chilled birch buds (Eagles and Wareing 1963).

The findings concerning changes in endogenous GA-like levels also indicate a control function in the removal of innate dormancy. The free levels of GA-like activity at R_f 0-0.2 increased during chilling perhaps reaching a critical level at some point. That this was interconverted is suggested by the high levels of a different GA-like compound during active growth.

Exogenous gibberellin applications have been effective in relieving dormancy of woody plant buds (Wareing and Saunders 1971), and turions of several aquatic angiosperms. For example, in *M. verticillatum* dormant turions could be caused to sprout by GA_3, kinetin and benzyladenine (Weber 1976a). This author reported that the cytokinins were most effective but even they required up to three weeks for a response. Recently formed turions of *M. exalbescens* were observed by the present author to be responsive to GA_3 but not to kinetin. The sprouting response obtained in this experiment occurred within 2 days. Turions of *Hydrilla verticillatum* also exhibit a significant sprouting response to GA_3 (Steward 1968). Turions of *U. vulgaris* were found to be totally unresponsive to GA_3 in terms of sprouting. As mentioned above, GA_3 did seem to have an effect in maintaining vegetative growth in plants obtained from innately dormant turions.

While endogenous cytokinins were not consistently detected it is important that they are detected at certain points during the study to at least indicate their presence. However, the most convincing evidence that there is a definite cytokinin involvement in dormancy control of *U. vulgaris* comes with experiments using exogenous application of kinetin. In such experiments kinetin caused immediate sprouting (within 24 hr) in turions collected during innate dormancy. Kinetin had no effect on growth maintenance in these plants and did not prevent turion reformation. The stimulation of sprouting by kinetin has been observed in turions of *Spirodela polyrhiza* (Czopek 1964 and Stewart 1969), and *Myriophyllum verticillatum* (Weber 1976a). In lateral buds of *Scabiosa, Coleus, Pisum* and *Helianthus* (Sachs and Thimann 1964) kinetin releases dormancy.

In summary, the results from determinations of endogenous levels are as follows: 1) ABA-like inhibitory activity is at a maximum just prior to dormancy induction, 2) levels of the ABA-like inhibitor are high throughout the period of innate dormancy and fall sharply prior to the onset of imposed dormancy, 3) levels of auxin-like and GA-like substances increase during dormancy, 4) high levels of a bound ABA-like compound correspond to a period of rapid growth, and 5) high levels of a bound GA-like compound are found preceding dormancy induction. The results of exogenous growth regulator experiments are these: 1) only kinetin can induce sprouting, 2) kinetin will not prevent reformation of turions in plants from turions collected during innate dormancy, 3) ABA will maintain turions collected during imposed dormancy dormant even at high temperatures, and 4) ABA will induce turion formation even under non-inductive environmental conditions. Preliminary experiments suggest that dormant turions released

by high temperature or kinetin treatment will grow and not re-enter dormancy if incubated with IAA and GA_3. Looking at these two sets of information the following situations of growth regulator control of dormancy in *U. vulgaris* emerge:

1.	High [ABA] Low [GA_n] Low [auxin]	=	Dormancy induction Dormancy maintenance
2.	High [ABA] High [cytokinin] Low [GA_n] Low [auxin]	=	Sprouting No further growth
3.	High [ABA] High [cytokinin] High [GA_n] High [auxin]	=	Sprouting Growth
4.	Low [ABA] High [GA_n] High [auxin]	=	Sprouting Growth

These sets of growth regulator combinations are intended to show the relationships between data from both experimental approaches. There are of course many other combinations possible and levels of endogenous growth regulators probably do not result in all or none responses. The growth regulator sets discussed in this report are not presented as building blocks that automatically result in a certain growth pattern. Instead they correspond best to actual growth manifestations that were observed. While some growth processes are dependent on a single growth substance many processes such as stolon and bud development depend on an interaction of growth substances (Wareing 1977). The importance of interactions of multiple growth substances was first stressed by Skoog and Miller (1957) in relation to the control of organ development in tissue cultures. Smith and Kefford (1964) postulated a model of bud dormancy based

on the interactions of a promotor (gibberellin) and an inhibitor. Khan (1975) has also proposed a useful model of primary, preventive and permissive substances. The combinations of these three classes of substances at physiologically effective levels regulate a particular developmental process (seed dormancy in his case). In this study the changing pattern of growth regulators result in changing potentials and manifestations of development found during that part of the life cycle of *U. vulgaris* defined as dormancy.

In *U. vulgaris* the correlations between environmental factors and growth regulator levels involved in developmental changes are summarized in Fig. 33. The changing patterns of growth regulators which occur in response to environmental cues resemble those which have been found for the induction and release of dormancy in woody plants. This study, using a different type of angiosperm, provide additional support for the hypothesis that dormancy in many diverse species is regulated in a similar fashion.

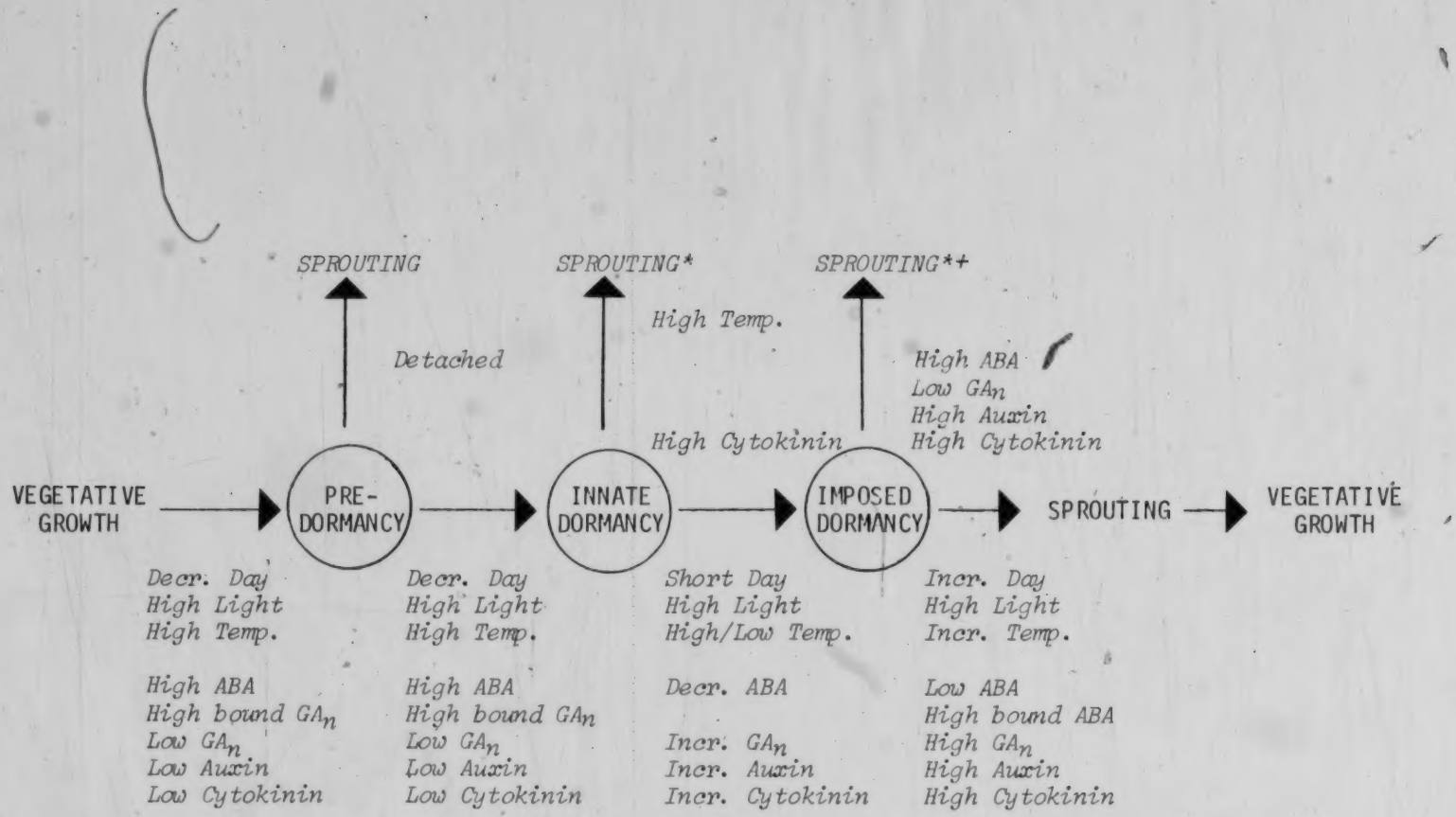

Fig. 33. Factors involved in the induction and release of dormancy in *U. vulgaris* turions. * - artificially induced sprouting that was not followed by vegetative growth. Increasing day and decreasing day mean increasing and decreasing daylengths respectively. + - turions collected during innate dormancy and treated to artificial conditions of low temp and no light.

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APPENDIX

An alternate extraction procedure and comparison to the modified Shindy and Smith Method

Early in this study an extraction protocol, based on a method for the identification and quantification of ABA via GLC analysis (Lenton, Perry and Saunders 1971) and of the general growth substance extraction method of Weber (1976b) was tested. This procedure was identical to the modified Shindy and Smith method (SS) through step 1A.

Following step 1A of the modified SS method, the alternate method called for adjustment of the aqueous phase pH to 2.8 with 2N HCl. The solution was partitioned 3X with equal volumes of diethyl ether. The aqueous fraction was retained for further extraction and the ether phase was reduced to dryness at 30 C under vacuum and the residue handled as in steps 4B and 5B of the SS method.

The pH of the retained aqueous phase was adjusted to 7 with 1N NaOH. This solution was refrigerated overnight at 2-3 C for alkaline hydrolysis of bound acids as in steps 5C, 6C and 7C of the SS method or the solution was extracted for cytokinins. If the latter was the case the pH was kept at 7 and the solution was treated as in steps 4A and 5A of the SS method.

The initial test of this extraction procedure was concerned mainly with the efficiency of ABA recovery. A known amount of synthetic ABA in distilled water solution was extracted using the alternate method and % recovery was determined by GLC analysis. As can be seen in Fig. 34 approximately 100% recovery resulted. Spiking a plant extract with a known amount of ABA followed by GLC analysis was unsuccessful due to numerous compounds which were unresolved from ABA. An attempt to recover spiked ABA in a plant extract by bioassay was also unsuccessful so the method was abandoned. In addition to

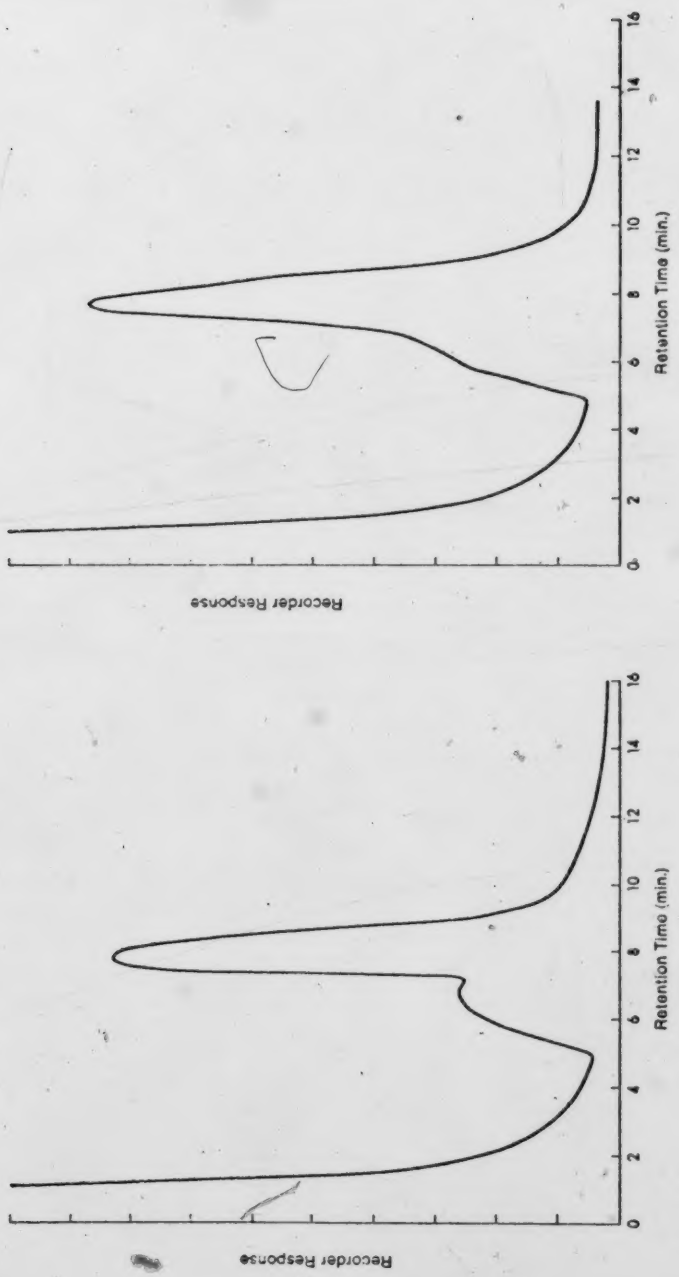

Fig. 34. Gas chromatograms of equal amounts of silylated synthetic ABA which had not (left) and which had (right) been carried through the alternate extraction procedure.

this difficulty with the alternate method a review of the literature revealed potential problems in recovery of other growth substance classes. The alternate method used an acidic ether extraction for the free acids (ABA, GA, IAA) with the cytokinins supposedly remaining in the aqueous phase. This procedure has been reported previously using diethyl ether (Hewett and Wareing 1973) and ethyl acetate (Henson and Wheeler 1976, van Staden 1973) to remove acidic inhibitors from solutions to be bioassayed for cytokinins. However, cytokinins (using kinetin as a model cytokinin) have been reported to partition into acidic diethyl ether and acidic ethyl acetate in significant amounts (Hemberg and Westlin 1973). Also, gibberellins are reported to partition into ethyl acetate more efficiently than into diethyl ether (Durly and Pharis 1972).

Using isotopically labeled synthetic growth substances (GA_3, IAA, kinetin and ABA) Shindy and Smith (1975) reported their method obtained 77% recovery of IAA, 83.5% recovery of ABA, 70.2% recovery of kinetin and 80% recovery of GA_3. No recovery information was provided for the alternate method. Using bioassays to determine percent recovery the results obtained in this study for the two extraction procedures is shown in Table 11. It must be noted that the spiking experiment using the alternate method involved use of tissue containing an inhibitor in the acidic ether fraction which co-chromatographed with IAA and GA_3. The values for the SS method were obtained from a spiked extraction of tissue not containing this inhibitor. An additional spiked extraction using the SS method and tissue containing high levels of this acidic inhibitor gave values of 35% recovery of GA_3 and 11% recovery of IAA. With the exception

Table 11. Per cent recovery of synthetic growth substances added to tissue extracts as determined by bioassay.

	Alternate Method[1]	SS Method[2]	SS Method[1]
IAA	8.3	121	11
GA_3	60	80	35
ABA	0	76	99
Kinetin	20	8	NA

[1] Tissue contained endogenous inhibitor co-chromatographing with IAA and GA_3.

[2] Tissue did not contain endogenous inhibitor co-chromatographing with IAA and GA_3.

of kinetin the recoveries found in this study for the SS method are comparable to those reported. The primary modification of the original SS method was the use of PVP for further purification via the selective removal of compounds with aromatic hydroxy groups (Glenn, *et al.* 1972). It was possible that this modification was causing the great loss of added kinetin. Although use of PVP for the purification of cytokinin extracts has been reported previously (Hewett and Wareing 1973) this possibility was tested. A kinetin spiked sample was divided; with one half slurred with PVP. As can be seen in Fig. 35, the PVP treatment enhanced biological activity by a factor of 10. The conclusion is that the extraction procedure renders kinetin biologically inactive, rather than providing incomplete separation since Shindy and Smith (1975) showed 70% recovery of label.

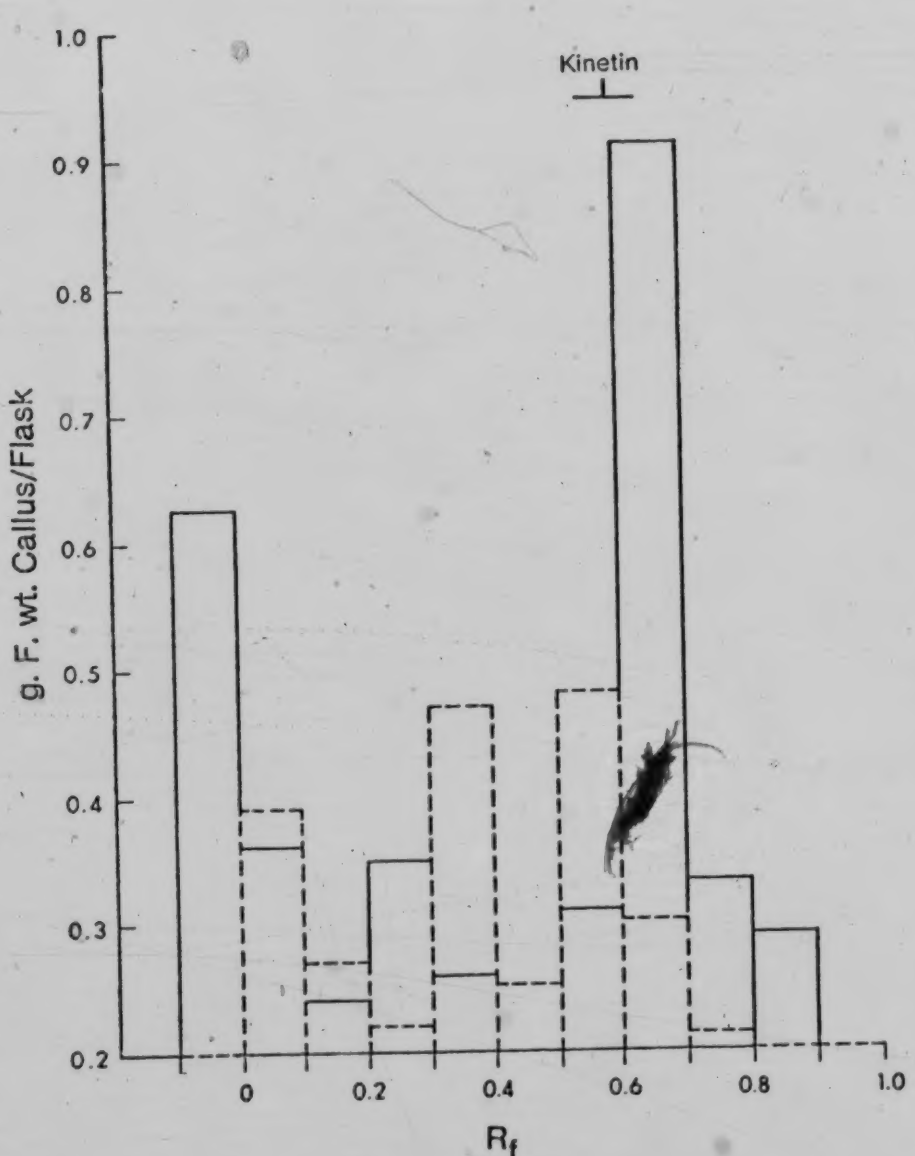

Fig. 35. Bioassay results of equal amounts of kinetin which had been carried through the Shindy and Smith extraction procedure with (—) and without (--) the PVP purification step.